Clinical and Laboratory evaluation of micro inflammation in patients on Haemodialysis

A dissertation submitted to the Tamilnadu Dr. M.G.R. Medical University in partial fulfillment of the University regulations for the award of D.M. (Branch – III) (Nephrology).

AUGUST 2011

BONAFIDE CERTIFICATE

This is to certify that the work presented in this dissertation titled "**Clinical and Laboratory evaluation of micro inflammation in patients on Haemodialysis"** done towards fulfillment of the requirements of the **Tamilnadu Dr. M.G.R. Medical University, Chennai for the D.M. (Branch–III) (Nephrology)** exams to be conducted in August 2011, is a bonafide work of the candidate **Dr.Jigy Joseph,** Senior Post graduate student in the Department of Nephrology, Christian Medical College, Vellore under my guidance and supervision. This dissertation has not been submitted, fully or in part to any other board or University.

Guide & Head of Department

Dr**.** V Tamilarasi M.D.,D.C.H, D.M.,
Professor and Head,
Department of Nephrology,
Christian Medical College,
Vellore – 632004

Acknowledgement

I thank God Almighty for making me able and providing me with right opportunities and right people at the right time. This dissertation would not have been possible without the support, encouragement, timely help and advice from many people.

I am greatly indebted to **Dr. V Tamilarasi,** Professor and Head, Department of Nephrology, Christian Medical College, Vellore for being instrumental in initiating this research venture, for her valuable inputs into the topic, and guidance through out the study.

I owe a deep sense of gratitude to **Prof Dr Chakko k Jacob** and **Dr George T John** former heads of our department for their encouragement in fulfilling the dissertation.

My special thanks to **Dr Madhivanan S** who deliberately extended his hearty co operation in preparation of this dissertation.

I extend my sincere thanks to **Dr .Basu G** for his constant suggestion and guidance at every step of data analysis.

It gives me great pleasure to place on record my obligation to **Dr Santosh Varughese, Dr Anjali Mohapathra** for their interest and unstinted support.

My special thanks to **Dr Vijayakumar** for his constant interest in helping out the laboratory tests

I am very much grateful to all the office staff for their timely help and support.

I am very much thankful to my wife **Dr Lopa** and son **Lejoy** for their inspiration and support towards the smooth completion of the dissertation.

I acknowledge the efforts of staffs of dialysis unit and the department of microbiology for making me able to collect the data for thesis.

I express my sincere gratitude to all the patients who were part of the study.

I would like to thank my colleagues for their help and support.

Abstract

Objectives:

To assess the correlation of the comorbid illness and inflammatory markers with outcome of patient initiated on maintenance dialysis.

Patients and Methods:

This is a prospective observational study on end stage renal disease patients initiated on maintenance haemodialysis in department of nephrology, CMC Vellore, from July 2009 to December 2010. Patients with less than 6 months of follow up, or on treatment with anti inflammatory drugs, or positive for hepatitis B or C virus or HIV were excluded from this study. Patient was divided into 3 groups based on the comorbidities according to Davies comorbidity scores. The comorbidity score for each patient was calculated depending on the number of following domains: diabetes mellitus, malignancy, ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, systemic collagen vascular disease, and other significant pathology. The grade of comorbidity was obtained from the total scores. Grade 0 is zero score, Grade 2 is score of one or two, and Grade 3 is score of three or more. Information about comorbid illness was obtained from clinical examination, medical records, lab parameters, ECG and echocardiography. Baseline lab tests including hsCRP and IL6 samples were taken before the initiation of maintenance dialysis. Patients were treated with thrice weekly standard bicarbonate dialysis and their clinical events were recorded accurately. Laboratory parameters were repeated every third month during the follow up period. Patients were prospectively followed up during the study period.

Results:

The mean age at initiation of maintenance dialysis was 52.72 ± 11.25 and males constituted the majority of patients (77.5%). Mean BMI of the patients was 21.15 ± 2.44. Diabetic nephropathy was the commonest native kidney disease (47.5%) followed by unknown native kidney disease in 35% of patients. Hypertension was present in 92.5% at the time of initiation of haemodialysis. Mean eGFR at initiation of dialysis was 5.66 ± 2.29 ml/min/BSA. Median follow up period was 310 (180 – 546) days. Hypotension was the commonest clinical event during follow up. About 25% of patients had one or more infection episodes with sepsis and access related infection being the commonest cause. 40% of patients had one or more episode of AV fistula failure during follow up of which 50% was due to primary AVF failure. During follow up death occurred in 10% of patients with cause of death being infection related in majority of patients followed by cardiac cause. Associated comorbid illness and baseline inflammatory markers IL6 and hsCRP were significantly higher in this group of patients.

Conclusions:

Comorbid illness and higher baseline inflammatory markers is a poor prognostic marker in maintenance haemodialysis patients. Measurement of baseline inflammatory markers level should be considered for patients initiating on maintenance dialysis for risk stratification and prognostification.

Introduction

Chronic inflammation is highly prevalent in end stage renal disease and is a major contributing factor for high mortality and morbidity in this population. Chronic inflammatory state will leads to endothelial injury which is considered as critical event in pathogeneses of atherosclerosis. Persistent inflammation in these patients is associated with various complications like cardiovascular mortality, atherosclerosis, malnutrition syndrome, higher hospitalization rates (1). Recent attention has focused on the inflammatory state as the cause of progression of underlying comorbid illness which leads to increased mortality in end stage renal disease. Reactivation of inflammatory cascade during the dialysis is associated with decreased survival (2). Inflammation plays a major role in arterial damage of dialysis patients. Underlying disease, retention of inflammatory mediators, auto-oxidation products, repeated exposure to dialysis filters and low grade infections are the probable factors for the inflammatory state.

Acute phase reactants include a class of proteins like C-reactive protein which are secreted mainly by hepatocyes under stimulation of various cytokines like interleukin 6. Inflammatory markers like C-reactive protein and interleukin 6 is elevated in dialysis patients. C-reactive protein is having a direct proinflammatory effect on human endothelial cell by inducing adhesion molecule expression. Interleukin 6 is a potent proatherogenic cytokine which can be demonstrated by the increased expression of interleukin 6 at the fibrous plaque stage of the atherosclerosis process (3). The measurement of these inflammatory markers might be useful for predicting survival in dialysis patients. Whether severity of inflammation as measured by these markers will gives prognostic information on patients on dialysis in our population is not yet studied.

In the present prospective observational study, we analyzed the clinical and laboratory parameters of patient initiated on maintenance haemodialysis in our institution and followed up to assess the correlation between the comorbid illness and inflammatory markers with outcome.

Review of Literature

End – stage renal disease is associated with high morbidity and mortality rates (4) . Even with improvement in dialysis technology the mortality and morbidity in end stage renal disease is very high. Cardiovascular disease is the main cause of mortality in end stage renal disease. The mortality of patients on dialysis is very high which is about 10-20% when compared with that of general population even when adjusted for age, gender and other co morbidities(5). The cardiovascular mortality accounts for about annual mortality of 9% which is about 10 to 20 fold higher than general population (6). Inflammation strongly predicts the all cause and cardiovascular mortality in patients on dialysis.(7). About 30 to 50% of patients on dialysis have elevated level of inflammatory markers.

Renal insufficiency is associated with accelerated atherosclerosis. Ishimura et al has shown that renal failure is a significant and independent risk factor for carotid atherosclerosis (8). The pathogenesis of atherosclerosis in end stage renal disease patients can be well explained by Ross theory (9). Endothelial activation is induced by any insult or risk factor which affects the vascular endothelium. This will leads to expression of E – selectin and adhesion molecules which include intracellular adhesion molecule -1 and vascular adhesion molecule -1.Macrophages and monocytes will be attracted to the altered endothelium. Later they will infiltrate the vessel wall and will initiate the inflammatory process. Monocytes will produce both pro and anti – inflammatory cytokines. The pro inflammatory cytokines includes interleukin-1β, IL-6, and tumour necrosis factor α. They induce the synthesis of acute phase reactant, C – reactive protein from the liver. CRP is both cardiovascular and direct risk factor for atherosclerosis. On activation of IL-6 sensitive sequence in the fibrinogen gene promoter, fibrinogen is produced. Fibrinogen links the inflammation to clot

formation. More than half of the patients on dialysis is having elevated plasma fibrinogen levels (10).

Causes of inflammation:

The causes of inflammations in patient on haemodialysis are multifactorial and includes patient related factors and haemodialysis related factors (See table 1).

Table 1. Causes of inflammation in hemodialysis

Patient related

- Underlying disease
- Comorbidity , Peripheral vascular disease
- Oxidative stress
- Ca × P metabolism (Calcification, Fetuin – A)
- Infection (apparent and non – apparent)
  - Helicobacter Pylori
  - Chlamydia Pneumoniae
  - Peridonditis
  - Tuberculosis and others
  - Vascular access
- Immunologic
  - Genetic
  - Nonfunctioning kidney transplants
- Encapsulating peritoneal sclrosis
- Anemia (hepcidin)
- Heart failure
- Obesity
- Tumors
- Physical exercise – sedentary life style

Dialysis technique related

- Retention of inflammatory mediators
- Oxidative imbalance
- Acetate
- Pyrogenic substances of the dialysate
- Complement activation, membranes and other material

Inflammation in dialysis patients may be due to renal failure itself or may be a consequence of dialysis or may be unrelated to both ie infectious cause. About 15% of deaths in end stage renal disease are attributable to infectious cause.

Renal failure:

Patients with renal failure are having elevated level of inflammatory markers even before initiating dialysis. Stenvinkel has shown from various studies the prevalence of elevated C reactive protein of about 35% in patients with renal failure without dialysis and more than 50% in patient on dialysis (11). Kidney is the major site of elimination of cytokines. Accumulation of inflammatory mediators in renal failure can results in inflammation. Reduced elimination of inflammatory mediators can lead to accumulation of factor D which is the rate limiting step in complement activation and amplification of C3 activation. Residual renal function is having an important role in inflammatory process. Bolton et al has shown creatinine as a major determinant of interleukin 6 level in renal failure patients (12). Elevated plasma levels of TNF-α and soluble TNF receptors in renal failure is correlated with severity of renal failure and glomerular filtration rate(13). Descamps Latscha et al demonstrated that there is a significant increase in plasma levels of IL 1 receptor antagonist from the earliest stage of renal failure. Spontaneous and lipopolysaccharide induced production of IL6 and IL1 in whole blood is high in dialysis patients when compared to general population.

Acidosis in renal failure can contribute to inflammation and results in protein catabolism and loss of lean body mass. Fluid overload state in renal failure may contribute to inflammation and is having high concentration of several cytokines. Edematous patients are found to have elevated endotoxin levels in plasma. There is negative correlation between volume overload and serum albumin in patients on

dialysis. It is proposed that volume overload leads to increase in endotoxin and cytokine levels which results in reduced albumin synthesis. Attaining dry weight in patients on dialysis can ameliorate inflammatory response.

Underlying comorbidity :

Associated comorbidity like heart failure, ischemic cardiomyopathy, diabetes mellitus, peripheral vascular disease can be a risk factor for inflammation and oxidative stress(14). Heart failure is associated with up regulation of cytokines. Elevated sympathetic activity in heart failure leads to enhanced cytokine response. TNF which is not expressed in normal myocardium is over expressed in response to increase in left ventricular pressure and volume overload. Reduction in TNF levels is documented with treatment with β blockers (15).

Postsynthetic modification of proteins:

Renal failure is associated with accumulation of proinflammatory compounds and products of metabolism. Advanced glycation end products(AGE) and advanced lipoxidation end products are increased in patients with renal failure. Kidney plays an important role in the metabolism of advanced glycation end products. AGE can leads to inflammatory response by activating mononuclear cels. Inflammation also can also increase the production of AGE. There is a correlation between the levels of advanced glycation products and cytokines with severity of renal failure (16).

Oxidative stress:

Renal failure decreases plasma antioxidant activity (17). Levels of IL 6, IL 1 and TNF α are elevated in patients with renal failure. The increase in accumulation of uremic solutes as renal failure progress can serve as a targets for increased oxidation. The loss of antioxidants like zinc, selenium, vitamin C, vitamin E can occur due to renal failure or as a result of dialysis and can leads to increased susceptibility to

oxidative injury. In renal failure there are decreased levels of plasma glutathione peroxidase activity. Dialysis can exacerbate oxidative stress. When compared with healthy individuals neutrophils obtained from patients on dialysis exhibit a higher rate of spontaneous production of reactive oxygen species. Oxidative stress can leads to lipid peroxidation and oxidative alteration of lipoproteins (18). Intravenous iron therapy in uremic patients is a contributing factor for oxidative stress by the production of strong oxidant hydroxyl radical (19).

Genetic factors:

Genetic factors play a role in inflammation in renal failure. Genitic factors like single nucleotide polymorphisms may significantly influence the immune response, level of inflammatory markers and prevalence of vascular calcification in patients with chronic renal failure. Studies have shown that genetic variation in the TNF-α 308 and IL 10 -1082 single nucleotide polymorphisms is associated with adverse clinical outcome in patients with end stage renal disease (20).

Dialysis Membrane:

During dialysis exposure of blood to bioincompatible dialysis membrane will cause activation of circulating mononuclear cells and can leads to induction of inflammation(21).Biocompatibility involves coagulation, thrombocytes, leucocytes, complement activation which results in cytokine and bradykine production and can leads to inflammation. Kaizu et al have shown that dialysis membrane properties predict the serum interleukin 6 levels in patients. Some investigators have suggested that even dialysis with biocompatible membranes may pose risks for activation of inflammation. Interaction of circulating nuclear cells will directly stimulate cytokine production is suggested by the fact that during in vitro dialysis of whole blood there is enhanced cytokine production(22). Dialysis results in altered mononuclear cells which

respond more vigorously to subsequent exposure to endotoxin. Activated complement factors like C3a and C5a will be increased during dialysis. There is significant difference in complement activation with various types of membranes with cellulosic membrane activates more and synthetic membrane activates less complements. Memoli et al has shown a significant differences in the plasma levels of CRP, IL 6 and albumin depending on the types of membrane used like cuprophan, synthetically modified cellulosic membranes and cellulose diacetate(23). Cells which leave cuprophan dialyser express large amounts of mRNA for IL 1β and IL 6 than the non complement activating membranes. These activated cells when stimulated subsequently with endotoxin ,they are sensitized to produce more cytokines.

Reuse techniques and number of reuse may also contribute to the interaction of blood with dialyser and can lead to changes in acute phase response. Pyrogenic reactions in the absence of septicemia are usually closely associated with reuse (24). Not only the type of membrane ,but also its flux and convective transport also may influence the inflammation. Cytokine induction on the blood side of the membrane is the result of complement activation, the permeation of bacterial products from the dialysate and direct blood membrane interactions. More pro inflammatory products will be removed and passage of bacterial products is hindered with high flux membranes and convective therapies, resulting in less inflammation on the blood side.

Dialysate Quality:

The water quality used for dialysis can be a contributing factor for inflammation. Bacterial cytokine inducing substances can be transferred from dialysate to the blood compartment. Cytokine inducing substances consists of a mixture of bacterial products. Bacterial products like LPS, exotoxins, and peptidoglycans can induce cytokines and activate immune functions. These

substances can be detected by the biological tests of cytokine induction in peripheral blood mononuclear cells. It is documented that use of less than sterile dialysate or back leakage of lipopolysaccharide through the dialysis membranes can results in dialysis related inflammation.

Use of ultrapure endotoxin free water by membrane filtration of dialysate is associated with reduced levels of cytokines(25). These findings suggest that either the monocytes may be activated by endotoxin that is in the dialysate side of membrane or the endotoxin can directly cross the dialysis membrane. The crossing of the endotoxin through the dialysate membrane may be of more importance with the use of highly permeable membranes. When using a high flux membranes types with more permiability the use of cytokine inducing substances free dialysate is essential and supplementary measures in addition to ultra filtration may be required. Bacterial derived short DNA fragments can also pass through the dialyser membrane. The peripheral blood mononuclear cells will ingest bacterial DNA. The unmethylated cytosine guanosine mofits in bacterial DNA allows phagocytic cells to recognize and to be activated. The cytokine inducing bacterial oligonucleotides are significantly small size to pass through the dialyser membranes and are not easily removed by conventional ultra filters.

The fact that majority of patients on dialysis do not exhibit evidence of activation of the inflammatory response despite exposure to dialysis membranes and dialysates points towards the factors other than the dialysis which are specific to individual patients.

Infectious cause:

About 15% of death in patients with end stage renal disease is attributed to infectious cause. Dialysis patients are at increased risk of infection as a consequence

of impaired humoral and cellular immunity and vascular access(26). Longitudinal cohort study using baseline data from the United States Renal Data system has shown that in a 7 year period 11.1% of nondiabetic patients and 12.5% of diabetic patients experienced at least one episode of septicemia (27). In this study the risk factor predicting septicemia were low serum albumin and advancing age.

Infection of the access site is very frequent and is often overlooked. Foreign materials in the access sites are especially liable to infection and can act as a source of bacteraemia. Diagnosis requires a high index of suspicion since physical signs may not be always present and even may require In^{111} white blood scans or other procedures. Venous catheters are associated with increased rates of infections when compared with other vascular access. Pastan et al had shown that medium term mortality and morbidity due to infections are well correlated with the use of venous catheters(28). Patients with clotted vascular access are having elevated inflammatory markers and may play an important role in the inflammatory process.

Dialysis patients are also subjected to other sources of infections like tuberculosis especially extrapulmonory as a consequence of decreased cellular immunity. Peridontitis is frequent in dialysis patients and is a common occult sourse of chronic inflammation that could lead to atherosclerosis and resistance to erythropoietic agents. Periodontal treatment can results in reduction in inflammatory markers (29). Associated diabetes mellitus was found to be risk factor for increased infection in dialysis population. Subclinical infections by microorganisms such as Chlamydia pneumoniae, herpes virus and combination of other factors can induce a micro inflammatory state in uraemic patients. Uremic patients with fluid overload endotoxin penetrate the intestinal mucosa and gains access to systemic circulation.

Consequences of micro inflammation:

Malnutrition:

Despite improvement in dialysis technique and patient care the mortality is still high in dialysis patients when compared to general population. Previously it was believed that factors related to dialysis treatment and technique were the main cause of poor clinical outcome. The multicenter randomized clinical trial, HEMO study failed to show any improvement in mortality or hospitalization by increasing dialysis dose or by using high flux dialysis membranes (30). Patients on maintenance dialysis with high rate of hospitalization and mortality, malnutrition and inflammation continue to be a major cause. Epidemiological studies have shown a strong association between malnutrition and inflammation in dialysis patients. In view of these two conditions coexisting in individuals with end stage renal disease the terms malnutrition – inflammation complex syndrome (MICS)(31) or malnutrition inflammation and atherosclerosis (MIA)(32) syndrome have been proposed.

Protein energy malnutrition is defined as the state of decreased body pools of protein with or without fat depletion, caused at least partly by inadequate nutrient intake to nutrient demand and or which is improved by nutritional replenition. Protein energy malnutrition is common in dialysis patients and is risk factor for poor quality of life and increased morbidity and mortality including cardiovascular death. The prevalence of protein energy malnutrition in dialysis patients varies from 18 % to 75% depending on the type of dialysis modality, nutritional assessment tools, and origin of the patient population (33). There is a good overlap in causative factors between malnutrition and inflammation in dialysis patients. The protein energy malnutrition appears to precede dialysis treatment and worsens progressively as the glomerular filtration decreases.

The development of protein energy malnutrition in end stage renal disease is secondary to inflammation. Pro inflammatory cytokines like tumor necrosis factor α can promote catabolic process by engendering protein degradation and by suppression of protein synthesis. They can also induce anorexia. Low appetite in dialysis patients has been associated with increased levels of inflammatory markers(34).Even in patients with intact appetite, are reported to develop weight loss and negative protein balance when there is associated inflammation due to shift in protein synthesis from muscle to acute phase proteins as renal function declines. In chronic kidney disease patients albumin synthesis is suppressed when CRP levels are elevated in serum. Inflammation can also lead to hypocholesterolemia which is a strong mortality risk factor in dialysis patients and a marker of poor nutritional status.

Anemia:

Inflammation is associated with anemia and erythropoietin resistance. Studies have shown association between anemia and inflammation in dialysis patient which is reflected by high levels of CRP or other proinflammatory cytokines as IL6 and TNF α. Serum levels of CRP, IL6 and TNF α is having a strong correlation with EPO doses. Stimulated mononuclear cells from dialysis patients will release numerous inflammatory cytokines such as IL 6, IL 1, TNF α and INF γ that may contribute to erythropoiesis suppression. The exact mechanism for this is not clear. Induction of apoptosis in erythroid progenitor cells is considered to be an important factor. Macrophages activated by inflammatory signals results in accelerated disposal of erythrocytes, shortening the life span of erythrocytes and thus decrease the HB concentration.

Hepcidin has been implicated in patients with end stage renal disease as a complementary mechanism for development of anemia. Hepcidin is an

antimicrobial peptide synthesized in the liver. Hepcidin inhibits intestinal absorption of iron and is released to circulation from macrophages. Hepcidin synthesis is stimulated by iron overload, hypoxia and inflammation with transcription induced by IL 6(35). Hepcidin may be considered as link between inflammation and anemia, acting as an indicator of functional iron deficiency (36).

Lactoferrin is present in polmorphonuclear leukocytes. It act as a iron scavenger with bactericidal activity (37). During inflammation as apart of acute phase reaction lactoferrin synthesis increases and can bind large amount of free iron. Iron bound to lactoferrin is taken up by activated macrophages which express specific receptors for lactoferrin. During inflammation this can leads to iron deprivation of erythroid precursors, which is not having lactoferrin receptors.

Cardiac effects:

Cardiovascular pathology is the major cause of death in end stage renal disease. The incidence of cardiac death is about 5 to 10 times greater in uremic patients than in age matched general population. Cardiovascular death can be due to myocardial ischaemia, heart failure and sudden death. Approximately 40% of cardiac death are due to myocardial ischaemia. In the Canadian multicentre study of 432 patients started on dialysis and prospectively followed for mean duration of 41 months clinical signs of cardiovascular involvement were very frequent. This study showed heart failure 31%, coronary insuffiency 15%, angina 19%, arrhythmias 7%, and peripheral vascular disease 8% in the dialysis population (38).

There are various cardiac effects of chronic inflammation in uraemic patients. The cardiac effects are summarized in table 2

Table 2 : Cardiac effects of chronic inflammation
Accelerated atherogenesis Instability of the plaque Direct myodepressant activity Increased deposition of ground substance Decreased number of myocytes Cardiac fibrosis Increased heart size

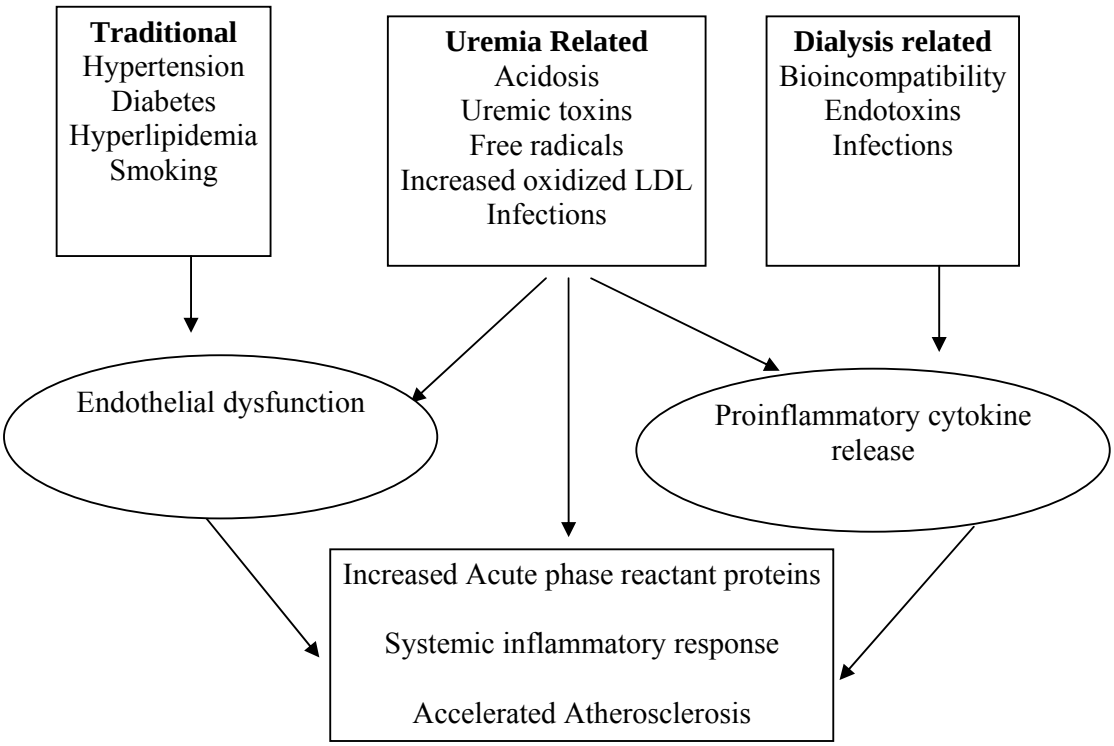
Several studies have shown the association of acute phase reactant proteins with cerebrovacular disease and ischaemic heart disease. The serum concentration of C reactive protein reflects the activity of cytokine mediated inflammatory process and is roughly proportional to extend of tissue injury. Owen et al in his studies on CRP levels in dialysis patients had shown that 35% of patients had values exceeding the upper limit of the reference range (39).

The excessive risk of cardiac diseases and atherosclerosis in chronic uraemic patients is the result of interplay between renal and non renal factors as well as the associated comorbidites. In uraemia the risk factors that are accepted for cardiovascular diseases in general population like older age, hypertension, hyperlipidaemia, diabetes mellitus, physical inactivity are higher. In uraemic patients traditional vascular risk factors are added to other factors which are specific for uremia and dialysis.

Inflammation plays an important role in the development of cardiovascular damage by number of different mechanisms: ie metabolic, endothelial, and coagulative. Several studies have shown that inflammation per se may play an important role in the development of atherosclerosis and death from ischaemic heart

disease. As previously mentioned by Ross, atherogenesis should be considered as an inflammatory process.

Figure 1 : Risk factors for atherosclerosis in end stage renal disease



Stimulation of atherogenesis may be due to modification of lipids, hypercoagulation, complement activation, and endothelial dysfunction. Apart from the accelerated atherogenesis, other negative effects mediated by inflammation may be expressed at cardiac level.

Inflammation will leads to localized recruitment of neutrophils and monocytes. The demonstration of activated macrophages in the cap of atherosclerotic plaque has led to the opinion that they contribute to plaque rupture through effects on matrix metalloproteinases(40). Cytokines such as TNF α and IL6 affects the

endothelial function and induce the endothelial expression of chemokines and adhesion molecules. The effects of these cytokines on triglyceride metabolism will further impair the endothelial generation of nitric oxide as result of raised circulating concentrations of non esterified fatty acids. The effects of IL6 on platelets, fibrinogen and coagulation and of TNF α on the expression of plasminogen activator inhibitor by hepatocytes, endothelial cells and adipose tissue will lead to a procoagulant state.

The microvascularization abnormalities will favor the development of fibrosis at the level of heart leading to increased deposition of ground substances, decrease in number of myocytes, and an increment in left ventricular interstitial mass. These abnormalities will lead to decreased capillary density and increases the distance of oxygen diffusion and could induce cellular ischaemia. This can lead to clinical signs of coronary heart disease in uraemic patients without angiographic abnormality. The chronic inflammatory status can results in two major cardiac alterations, the coronary atheromatous vascular disease and myocardial damage which will results in uraemic cardiomyopathy.

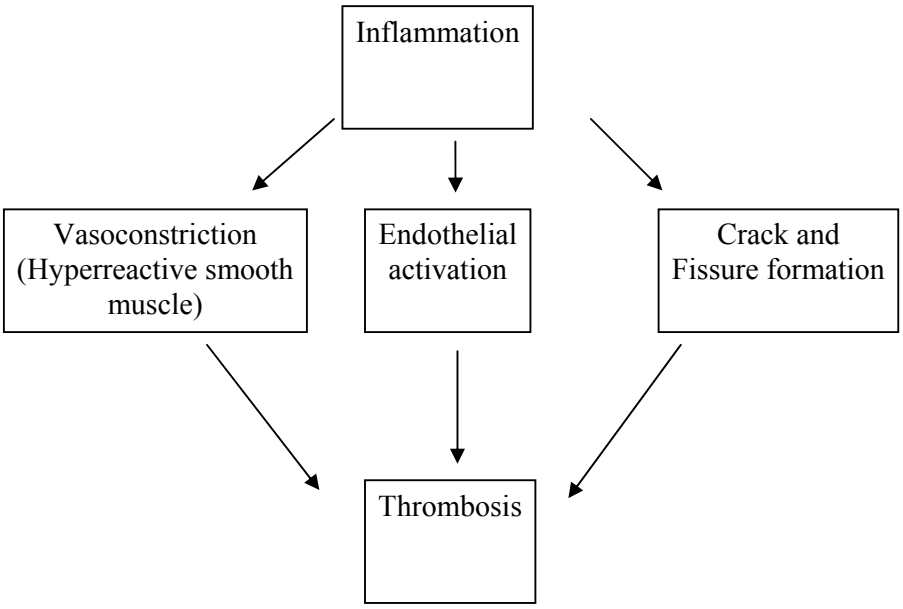
The prevalence of coronary artery disease in end stage renal disease is very high. About half of patients on maintenance dialysis have evidence of coronary artery disease before starting dialysis. Approximately half of the 40% patients who develop coronary events subsequently will do so in the first year of dialysis (41). There is increased frequency of complex calcified atheroma in the coronary artery of uraemic patients. Significantly calcified plaques have been shown in qualitative analyses of coronary arteries in patients with end stage renal disease. In uraemic patients the mean thickness of coronary artery is significantly higher when compared with normal population. Inflammation may modify the arterial wall and plaque morphology in uremic patients. Elevated plasma phosphate and increased calcium

phosphate products are associated with increased ectopic vascular and cardiac calcifications.

Inflammation can also leads to calcification of atherosclerotic plaque and apoptosis can occur in response to inflammatory cytokines. Soluble cytokines in the atheroma can trigger programmed cell death, along with the T cells which is also involved in eliminating some smooth muscle cells. Certain T cell in plaques can express Fas ligands on their surface. The Fas ligand can engage Fas on the surface of smooth muscle cell and along with the soluble pro inflammatory cytokines it can lead to death of smooth muscle cells (42).

Inflammation has a crucial role in thrombus formation. The release of inflammatory cytokines can induce endothelial activation with modification of the physiological properties of the endothelium and results in pro coagulant activity and vasoconstriction. The muscle cells will be hyperactive to the vasoconstrictive stimulus. There will be an increase release of metalloproteinases which will induce degradation of the fibrous caps, fissuration and exposure of the thrombogenic component of the lipid nucleus. These all will finally results in greater tendency of plaque instability and thrombus formation. Atheromatous plaques in uraemic patients are more vulnerable to rupture due to inflammation and fostering degradation of matrix covering the plaques. The impaired fibrogeneis associated with uremia can enhance this process. Uremic patients plaques are more instable than contol (41).

Figure 2 : Mechanism of thrombus formation in uraemia



Considering atherosclerosis coronary artery disease in uraemic patients as a consequence of an inflammatory disease will provides the basis for developing new insights into the pathogenesis of uraemic vascular and cardiac damage. The discovery of reliable inflammatory markers would be helping in the pre morbid diagnosis of atherosclerosis and could provide a potential therapeutic end point for disease activity.

Vascular calcification:

Vascular calcification is more common and even more severe in dialysis patients than in general population. The arterial intimal and medial calcifications can constitute a significant morbidity and mortality markers that is associated with coronary atherosclerosis and arterial stiffness. Causes of vascular calcification in dialysis patients are multifactorial. Altered mineral metabolism plays a important role. The usual factor is the increase in serum phosphate or calcium – phosphate product.

Inverse correlation between vascular calcification inhibitors such as fetunin A and matrix Gla protein and the inflammatory markers has (43)shown new insights to the pathogeneses of calcification in uraemia(43).

Insulin resistance:

Peripheral insulin resistance is features of end stage renal disease (44). Several studies have shown that hyperglycemia and hyperinsulinemia due to insulin resistance may be an important contributing cause of the premature atherosclerotic process in uraemic patients. Accumulation of some toxic uraemic toxins is considered to be the cause of insulin resistance (45). The proinflammatory and proatherogenic cytokines like TNF α and IL6 which is accumulating as the renal function worsens is considered as uraemic toxins for insulin resistance. Insulin stimulated storage of glucose is also decreased by TNF α . When TNF α was administered to animals it induced insulin resistance and once it was neutralized with thiozolidinediones there was improvement in insulin sensitivity (46).

It is documented that the features of insulin resistance syndrome such as increased body mass index, serum lipid levels and fasting glucose levels are linked to inflammation. Hak et al have shown that low grade inflammation and ICAM 1 are closely associated with insulin resistance in non diabetic elderly patients(47). Inverse relationship between insulin sensitivity and IL6 levels was shown by Fernandez Real et al (48). Management of low grade inflammation may be future potential target for treating insulin resistance in end stage renal disease.

Markers of Inflammation:

Recent attention has focused on the role of increased inflammation in promoting progression of underlying comorbid illness and thus leading to increased

morbidity and mortality in end stage renal disease patients. Studies have shown association between inflammatory markers and survival on dialysis(49). All acute phase reactants are markers of inflammation (see table 3).

Table 3 : Acute phase reactants

Positive acute phase reactants	**Negative acute phase reactants**
Proinflammatory cytokines IL – 6 TNF – α Other interleukins (IL – 1, etc) Other positive acute phase reactants CRP Serum amyloid A Ferritin Fibrinogen α 1 antitrypsin	Nutritional markers Albumin Transferrin or TIBC Prealbumin Cholesterol Other negative acute phase reactants Histidine rich glycoprotein

Residual renal function is having an important role in inflammatory activity. There is a significant increase in serum cytokine levels with the deterioration of renal function. A strong positive correlation between creatinine clearance and various cytokines has been shown in uderdialyzed patients with varying degree of renal failure(50). Reduced renal function in nephrectomized rat may affect clearance of TNF α and IL 1 and it has been suggested that proinflammatory cytokines actually could be considered as a uraemic toxin(51).

Albumin:

Hypoalbunminemia is a powerful predictor of death in dialysis patients. Hypoalbuminemia has been associated with about 20 fold increase in relative risk of death in dialysis population(52). There is a strong evidence that inflammation plays a important role in determining the serum albumin level in dialysis patients(53). Both

CRP and cytokine levels are predictive of temporal variation in albumin levels in dialysis patients in cross sectional studies and they also predict survival. Albumin is an important negative acute phase protein. Inflammatory conditions are associated with decresed hepatocyte synthesis of albumin mRNA in response to cytokines. The finding that hepatic synthesis of albumin is increased in dialysis rules out hepatic disorder as cause for hypoalbuminemia in dialysis patients. Prealbumin is a highly sensitive marker of nutritional status in view of its rapid turn over rate and short half life(54).

Ferritin :

Ferritin is usually used as a marker of iron status in dialysis patients. However serum ferritin is also an acute phase reactant which can be increased in inflammatory conditions. Dialysis is associated with increased inflammation and about 40 – 60% of dialysis population is having elevated inflammatory markers. Serum ferritin results from the leakage of tissue ferritin, an intracellular iron storage protein which have heavy and light subunits. The level of ferritin in plasma represents the balance between secretion and its clearance. The liver dysfunction and inflammatory conditions will interfere with synthesis and clearance of ferritin, resulting in increased serum ferritin levels which is not related to iron metabolism.

During acute phase response inflammatory cytokines will increase the synthesis of both H and L subunits of ferritin through an increased translation of preformed ferritin mRNA. This is usually associated with increased hepcidin activity(55). Inflammation induced hyperferritinemia may results in functional iron deficiency and can block iron mobility. This phenomenon may be useful in acute inflammation by iron containment in reticuloendothelial systems but harmful under chronic inflammation by causing refractory anemia in chronic kidney disease. In view

of since serum ferritin is an inflammatory marker, the diagnostic validity and reliability of serum ferritin in diagnosing anemia and iron treatment adequacy need to be reviewed.

C - Reactive protein (CRP):

C - Reactive protein (CRP) is a prominent inflammatory marker in dialysis patients. CRP is having a molecular weight of 115kDa with a pentamer structure. Function of CRP is not clearly defined, but may act as a clearance factor for endotoxin and opsonized bacterial products. CRP is 5 to 10 folds higher in hemodialysis patients than in general population and is clearly multifactorial in orgin. Elevated CRP is the consequences of elevated levels of circulationg proinflammatory cytokines. TNF α and IL1 isoform can stimulate the expression of IL 6 which leads to the augmented expression of the CRP gene in the liver. Oxidative stress due to oxidized low density lipoprotein and advanced glycation end products in dialysis patients will stimulates cells and endothelium to produce IL6 which in turn activate liver to secrete CRP and other acute phase proteins such as fibrinogen and lipoprotein (a) .

Several evidences are there showing that CRP per say may contribute to atherogenisis and may directly cause tissue damage. Cross sectional studies with single CRP values have shown that about 30 – 50 % of predialysis, haemodialysis, peritoneal dialysis patients have serological evidence of an activated inflammatory response with elevated serum CRP levels(56). In view of short half life , whether changes in CRP values which are associated with intercurrent clinical events, influence the long term prognosis of patient on dialysis needs to be established.

Proinflammatory cytokines :

Number of proinflammatory cytokines like TNF α, IL 6 and IL 1 and anti-inflammatory cytokines like IL 10 orchestrate the inflammatory reponse. Available data shows that IL 6 and its soluble receptor (sIL- 6 R) are central regulators of inflammatory response. IL 6 system can leads to inflammatory events through the activation and proliferation of lymphocytes, differentiation of B cells, leukocyte recruitment and induction of the acute phase protein response in liver (57).

IL 6 is a poypeptide with 22 to 27 kDa and is secreted from activated monocytes, macrophages, fibroblasts, adipocytes and endothelial cells in response to various stimuli such as TNF α, IL -1 β, bacterial endotoxins and oxidative stress. IL 6 acts via a receptor complex consisting of a specific IL 6 receptor and a signal transducing subunit (gp130).The soluble form of receptors reach the circulation by shedding and regulate the IL 6 activity. The binding of IL 6 to its receptor will increase the half life of IL 6 and extend the bioactivity of IL 6 to organs containing the gp130 membrane binding site. End stage renal disease is associated with elevated plasma levels of IL 6 due to loss of kidney function, fluid overload, oxidative stress, associated infections and dialysis related factors.

Studies have shown that proinflammatory cytokines have direct atherogenic properties. Injection of recombinant IL 6 in apolipoprotein E deficient mice led to exacerbation of early atherosclerosis suggesting that IL 6 could play a primary role in atherosclerosis (58) . IL 6 is primary stimulant of soluble intracellular adhesion molecule 1 (ICAM 1) which results in attachment and migration of leucocytes across the endothelial surface. The clinical effects of elevated IL 6 and strategies to reduce IL 6 should be evaluated to confirm the importance of this cytokine as a central mediator of inflammatory response in end stage renal disease.

Treatment strategies:

Even though end stage renal disease is associated with high prevalence of chronic inflammation, little is known about its management. Although epidemiological studies has shown poor outcome in patients with renal insufficiency there is no randomized control trials to show outcome improvement with nonspecific inflammation reducing modalities. There is no standardized treatment strategy for chronic inflammation in renal insufficiency. A general principle includes treatment of occult infection, correction of fluid overload and management of chronic heart failure and coronary heart disease.

Renin Angiotensin system inhibition:

Studies has shown that angiotensin converting enzyme (ACE) inhibitors can suppress the production of catabolic cytokines like TNF α and IL 1 in vitro in human monocytes and in vivo in mice(59). ACE inhibitors use in end stage renal disease is associated with lower levels of TNF α and CRP(60). Whether the reduction in inflammatory markers is due to a direct suppressive effect of ACE inhibitors on cytokine production or an indirect effect from amelioration of heart failure is not clear.

Apirin :

Aspirin is known to reduce the levels of CRP and IL 6 in patient with coronary artery disease. Even though use of aspirin for micro inflammation in end stage renal disease may be logical, the risk of bleeding should be considered. Generalized use of aspirin for treating micro inflammation in dialysis patient cannot be advocated until prospective randomized safety studies have been performed.

Statins :

Statins are shown to have anti-inflammatory properties and have demonstrated reduction in CRP levels in both renal and nonrenal patients. Anti inflammatory property may be due to their lipid lowering effects. It has been also proposed that inhibition of nonsterol compounds from mevolanate may also be responsible for the anti inflammatory effects (61). Statins have shown to reduce both cardiovascular death and all cause mortality death in dialysis patients(62) . Subsequent large clinical trails failed to show such benifit(63)(64).

Antioxidant :

Since oxidation products are mediating inflammation in end stage renal disease patients, use of antioxidants can modulate cytokine biology. Treatment with high dose viamin E supplementation reduced cardiovascular end point and myocardial infarction in dialysis patients(65). Use of vitamin E coated dialyzer membrane resulted in reduced release of myeloperoxidase , indicating a less neutrophilic activation(66). In another small study use of acetylcysteine reduced the cardiovascular events in dialysis patients (67) . It is unclear upto what extend the beneficial effects of anti oxidant treatment is due to its anti inflammatory properties.

Sevelamer :

Sevelamer hydrochloride is a cationic polymer which is used as a intestinal phosphate binder in patients with end stage renal disease .It is having pleiotropic effect of amelioration of inflammation. CRP levels was reduced on treatment with sevelamer(68).

Megesterol acetate:

Megesterol acetate is a synthetic derivative of progesterone and is used as an appetite stimulant. Megestrol acetate can inhibit the activity of IL1, IL6, TNF α

.The beneficial effects of using Megesterol acetate in dialysis patients are due to improvement in appetite, and increased dry weight and quality of life(69). The anti-inflammatory potential of Megesterol acetate in chronic kidney disease patient needs to be evaluated.

Bardoxolone methyl (RTA 420):

Bardoxolone methyl is an antioxidant inflammation modulator which inhibits immune mediated inflammation by redox homeostasis in inflamed tissue through the induction of cytoprotective transcription factor NrF2 and suppress the activities of pro oxidant and proinflammatory transcription factors. Bardoxolone has shown significant anti-inflammatory activity in various animal models inflammation in renal failure like ischemia reperfusion injury, renal damage in cisplatin model. Bardoxolone is currently under phase II clinical trial in assessing its ability to slow the progression of kidney disease in patients with advanced diabetic nephropathy.

Etanercept :

Etanercept is an anti TNF agent. In a small pilot study on dialysis patient with administration of Etanercept showed only a small improvement in pre albumin levels (70). The effectiveness of Etanercept in improving albumin and CRP levels in dialysis patient is under phase II randomized double blind placebo – controlled clinical trial.

Pentoxiphylline :

Pentoxiphylline is a non specific phosphodiesterase inhibitor which inhibits TNF transcription. The anti-inflammatory and anti oxidative nutrition in dialysis patients (AIONID) study which is sponsored by National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) is studying the effect of oral nutritional

supplements with anti-inflammatory and antioxidant therapy along with Pentoxiphylline in 100 patients receiving hemodialysis.

Anakinra:

Anakinra is a human recombinant IL2 receptor antagonist which is showing some promise in the treatment of inflammation in chronic kidney disease patients. Study on pharmacokinetics of anakinra in dialysis patients showed that dialysis is having very little effect in clearance and thrice weekly dosing may be possible (71). The safety and efficacy of ankinra in chronic dialysis patient is currently under randomized controlled clinical trial.

Optimal dialysis treatment:

Dialysis procedure itself can evoke inflammation and using biocompatible membrane and ultra pure dialysate can reduce the inflammatory response (72) . Optimization of dialysis can improve the inflammatory status of dialysis patient. However HEMO study failed to show a major improvement in outcome with the use of high flux membranes (73). This may be due to the fact that the membrane effect on survival may have been small compared to other variables. Optimization of dialysis modality should be still considered as an important step in reducing inflammatory response in dialysis patients.

Aims and Objectives

Aims:

1. To study the association between comorbidites and outcome in maintenance hemodialysis patients.
2. To asses the association between baseline inflammatory markers and outcome in maintenance haemodialysis patients.
3. To assess the correlation between inflammatory markers in maintenance hemodialysis patients.

Patients and Methods

This prospective observational study was conducted in the department of nephrology, Christian medical college, Vellore from July 2009 to December 2010. End stage renal disease patients who were initiated on maintenance haemodialysis in our dialysis unit during this period were recruited for study.

Inclusion Criteria:

1. Patients with end stage renal disease who are initiated on maintenance haemodialysis in our dialysis unit
2. Age ≥ 18 years

Exclusion Criteria:

1. Patients with other inflammatory conditions like chronic infection
2. Patients on treatment with steroids, NSAIDs and other antiiflammatory drugs
3. Patients positive for HBV, HCV or HIV
4. Patients on maintenance dialysis with less than 6 months of follow up.

Based on these criteria 40 patients who were initiated on maintenance dialysis were enrolled for the study.

Study design:

This is a prospective observational study. Baseline clinical parameters including a detailed history and lab parameters were taken when patient is started on maintenance haemodialysis. Associated comorbidites was noted. Patient was divided into 3 groups based on the comorbidities according to Davies comorbidity scores (74). The comorbidity score for each patient was calculated depending on the number of following domains: diabetes mellitus, malignancy, ischemic heart disease, peripheral vascular disease, left ventricular dysfunction, systemic collagen vascular disease, and other significant pathology. The grade of comorbidity was obtained from the total scores. Grade 0 is zero score, Grade 2 is score of one or two, and Grade 3 is score of three or more. Information about comorbid illness was obtained from history,clinical examination, medical records, lab parameters, ECG and echocardiography. Patients were treated with thrice weekly standard bicarbonate dialysis and the clinical events were recorded accurately. Laboratory parameters were repeated every third month during follow up period. Patients were prospectively followed up during the study period.

Laboratory parameters:

Blood sampling was done before the initiation of maintenance dialysis. Hemoglobin, WBC count, Total protein, serum albumin, urea, Creatinine, electrolytes, lipids, calcium, phosphate, parathyroid hormone(PTH), Ferritin, serum iron, TIBC were measured using standard laboratory methods. HsCRP was measured by commercial kit (cardiophase hsCRP, Siemens health care diagnostics, Marburg, Germany) by means of particle enhanced immunonephelometry. Sample for IL6, after centrifugation the plasma samples were stored immediately at minus 70^0 C until required for testing. IL 6 was measured using ELISA kits (BD OptEIA, BD Biosciences, San Jose, USA). Lab parameters were repeated every third month except for HsCRP and IL6 which was repeated at 6 th month.

Statistical analyses:

Data are expressed as mean ± SD or median with range. A p value of < 0.05 was considered as statistically significant. Comparison of differences of categorical variables between the groups was done by chi-square test or fishers exact test as applicable. Comparisons of continuous variables between multiple groups were performed by ANOVA test or independent sample tests as applicable. Patient survival was analyzed by Kaplan-Meier survival analysis and log rank test was used to study the difference in the effect of various factors on survival. Statistical analysis and graphics was done using SPSS version 15, Microsoft office excel and PowerPoint 2007.

Results

The Baseline clinical and laboratory profile:

The demographic profiles of the patients are shown in table 4. Male constituted about 77.5% of the cohort. Mean age was 52.72 ± 11.25 years. Mean BMI was 21.15 ± 2.44. Diabetes mellitus was present in 52.5 % and 92.5 % of patients were hypertensive at the time of initiation of dialysis. Smoking history was present in 17.5% of patients. Mean eGFR during initiation of dialysis was 5.66 ± 2.29. Mean inter dialytic weight gain was 2.46 ± 0.83. Median follow up period was 310 days (range: 180 to 546 days)

Table 4 : Demographic profiles of patients	
Age (years)	52.72 ± 11.25
Males	31 (77.5 %)
Smoking history	7 (17.5 %)
Diabetes mellitus	21 (52.5%)
Hypertension	37 (92.5%)
BMI (kg/m^2)	21.15 ± 2.44
Baseline GFR (ml/min/BSA)	5.66 ± 2.29
Inter dialystic weight gain (kg)	2.46 ± 0.83
Urine output (ml/day)	370 (100 – 1000)
Median follow up days	310 (180 – 546)

Native Kidney Disease:

Diabetic nephropathy was the commonest native kidney disease (47.5%) followed by unknown native kidney disease (35%), chronic glomerulonephritis (7.5%), Obstructive nephropathy (5 %), hypertensive nephrosclerosis (2.5%) and chronic interstitial nephritis (2.5%) (Table 5).

Table 5: Native Kidney Disease:	
Diabetic Nephropathy	19 (47.5%)
Unknown NKD	14 (35 %)
CGN	3 (7.5 %)
CIN	1 (2.5%)
Hypertensive Nephrosclerosis	1 (2.5%)
Obstructive nephropathy	2 (5 %)

Baseline laboratory profiles of patients are shown in table 6. Mean hemoglobin was 8.12 ± 1.70 gm/dl. Mean value of urea and Creatinine at initiation of dialysis was 192.59 ± 84.49 mg/dL and 11.25 ± 4.58 mg/dL respectively. The median value of PTH was 273 pg/ml (range 1 – 1425) at initiation of haemodialysis. The mean hsCRP value was 28.5 mg/L with median value of 14.5 and range from 0.79 to 132. The mean IL 6 value was 22.7 pgm/L with median value of 14.5 and range from 4.2 to 119.

Table 6 : Baseline characteristics of patients

Hb (gm/dl)	8.12 ± 1.70
Total leukocyte count(mm^3)	8758 ± 3010
Urea (mg/dL)	192.59 ± 84.49
Creatinine (mg/dL)	11.25 ± 4.58
Sodium (m mol/L)	132.6 ±7. 8
Potassium (m mol/L)	4.9 ± 1.2
Bicarbonate (m mol/L)	13.9 ± 4.2
Calcium (mg/dL)	7.9 ± 0.9
Phosphorus (mg/dl)	6.1 ± 2.9
PTH (pg/ml)	273 (1 – 1425)
Total protein (g/dL)	6.9 ± 0.8
Albumin (g/dL)	3.7 ± 0.5
Cholesterol (mg/dL)	164.8 ± 53.8
Ferritin (ng/ml)	269 (13.6 – 2068)
Serum iron (µg/dL)	48.5 (10 – 223)
TIBC (µg/dL)	207.1 ± 42
HsCRP (mg/L)	14.5 (0.79 – 132)
IL 6 (pgm/L)	14.5 (4.2 – 119)

Patients were divided into 3 groups depending on the comorbid illness according to davies score. The majority of patients were in grade 0 and 1 (45% in each group) and 10% of patients were in grade 2. The baseline characteristics of the three groups were similar except for statistically significant difference in age.

Table 7 : Baseline characteristics of patients according to Davies score

	Davies grade 0 (n = 18)	Davies grade 1 (n = 18)	Davies grade 2 (n = 4)	p
Age (years)	48.1 ± 12.7	55.7 ± 8.5	59.7 ± 7.8	0.049
Males	15 (83.3 %)	13 (72.2 %)	3 (75 %)	0.863
Smoking history	5 (27.8%)	1 (5.6 %)	1 (25 %)	0.167
Hypertension	17 (94.4 %)	17 (94.4 %)	3 (75 %)	0.443
Diabetes Mellitus	0	17 (94.4%)	2 (50%)	0
BMI(kg/m^2)	21.1 ± 2.6	21.3 ± 2.4	20.5 ± 2.5	0.720
Baseline GFR	4.7 ± 1.7	6.6 ± 2.6	5.2 ± 1.3	0.583
Inter dialytic wt gain	2.35 ± 0.9	2.46 ± 0.7	2.9 ± 0.7	0.219

The baseline lab parameters of patients according to davies score is shown in table 8.

Table 8 : Baseline lab parameters of patients according to Davies score

	Davies grade 0 (n = 18)	Davies grade 1 (n = 18)	Davies grade 2 (n = 4)	p for trend
Hb (gm/dl)	7.4 ± 1.5	8.3 ± 1.5	9.8 ± 1.9	0.088
Tc (mm^3)	8171 ± 3007	9184 ± 2940	9425 ± 3700	0.574
Urea (mg/dL)	194.1 ± 80.5	187.6 ± 75.2	208.5 ± 100.2	0.810
creatinine(mg/dL)	13 ± 4.6	9.6 ± 4.3	10.4 ± 2.2	0.128
Sodium(mmol/L)	134 ± 5.2	132.1 ± 9.5	128 ± 10.4	0.368
Potassium(mmol/L)	5 ± 1.1	4.6 ± 1.2	5.8 ± 1.5	0.370
Hco3 (m mol/L)	13.3 ± 3.7	14.5 ± 4.4	14.2 ± 6.5	0.844
Calcium(mg/dL)	7.5 ± 1.1	8.1 ± 0.7	8.6 ± 0.5	0.021
Phosphorus(mg/dL)	6.9 ± 3.1	5.1 ± 2.3	6.5 ± 3.1	0.635
PTH(pg/ml)	484 (24.4 -1425)	230 (13 – 781)	133 (1 – 349)	0.010
Total protein(g/dL)	7.1 ± 0.7	6.9 ± 0.9	6.6 ± 0.5	0.157
Albumin(g/dL)	3.8 ± 0.5	3.6 ± 0.5	3.3 ± 0.3	0.038
Cholesterol(mg/dL)	159.3 ± 59.6	180.5 ± 53.5	119.5 ± 21.5	0.148
Ferritin(ng/ml)	251 (64 – 2068)	281 (13 – 1650)	409 (105 – 976)	0.809
Serum iron(µg/dL)	49.5 (10 – 223)	51 (28 – 83)	29.3 ± 5.6	0.014
TIBC(µg/dL)	211 ± 46.2	209 ± 39.4	175.3 ± 26.2	0.122
HsCRP(mg/L)	29.7 (0.9 – 132.0)	12.6 (0.8 – 74.7)	43.6 (7.2 - 123.0)	0.399
IL 6(pgm/L)	7.8 (4.2 – 23)	16.1(5.7 – 45.4)	42.4(36.9–119.0)	0.003

Clinical events during follow up:

The clinical events that occurred during the follow up time were documented. Hypotension was the commonest event during maintenance dialysis followup. 21 patients (52.5%) had one or more episode of hypotension during follow up. 11 patients (27.5%) had one or more episodes of infections. Six patient had sepsis during follow up. One patient had tuberculosis. 13 patients (32%) had one or more episode of pulmonary oedema during follow up period. 12 patients (30%) required at least one blood transfusion. 16 patients (40%) had one or more episode of AV fistula failure of which 8 patients (50%) had primary AVF failure. 2 patients developed access thrombos which were operated. One patient had fracture Lt femur which was corrected surgically by open reduction and internal fixation. Four patients expired during the follow up period of which one death was due to cardiovascular cause and three were infection related deaths .

We analyzed the association between the baseline inflammatory markers and clinical events. Even though there was a trend to higher values of baseline inflammatory markers in clinical events like hypotension, LVF, pulmonary oedema, infections, AVF failure, blood transfusions, it was not statistically significant.

Analysis of clinical events with IL6 levels is shown in table 9. Higher values of IL6 were seen in patients with one or more episodes of clinical events when compared with those who were not having the similar events during follow up.

Table 9 : Clinical events and IL 6

EVENTS		**IL 6**	**p value**
Death	Yes	52.1 ± 47.4	0.007
	No	15.7 ± 11.1	
Hypotension	Yes	30.6 ± 31.2	0.065
	No	12.2 ± 8.7	
LVF & pulmonary oedema	Yes	36.5 ± 36.2	0.111
	No	14.2 ± 9.6	
Infections	Yes	38.5 ± 38.3	0.156
	No	14.8 ± 9.5	
AVF failure	Yes	25.4 ± 32.4	0.433
	No	14.4 ± 12.6	
Blood transfusion	Yes	32.1 ± 43.9	0.815
	No	19.0 ± 13.8	

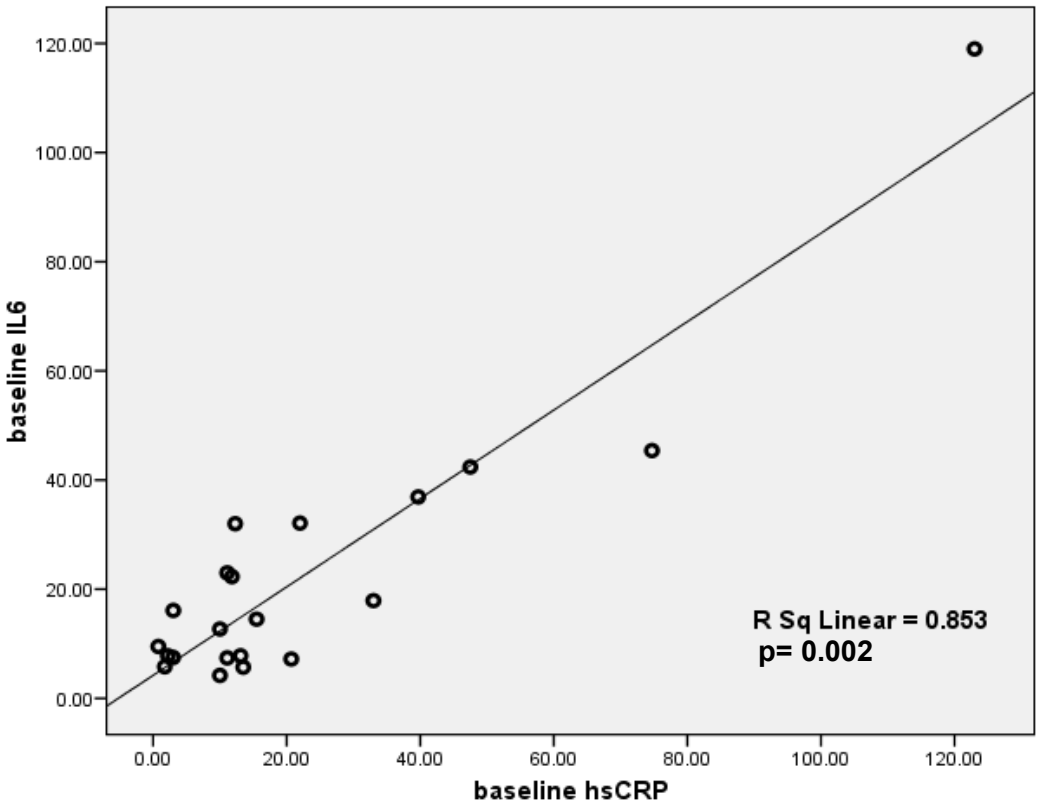
Analysis of clinical events with hsCRP levels is shown in table 10. Higher values of hsCRP were seen in patients with one or more episodes of clinical events when compared with those who were not having the similar events during follow up.

Table 10 : Clinical events and hsCRP

EVENTS		**hsCRP**	**p value**
Death	Yes	62.1 ± 48.2	0.038
	No	24.7 ± 31.3	
Hypotension	Yes	24.2 ± 29.5	0.394
	No	33.3 ± 39.6	
LVF & pulmonary oedema	Yes	45.6 ± 45.4	0.091
	No	20.2 ± 24.9	
Infections	Yes	42.4 ± 44.7	0.148
	No	22.5 ± 28.0	
AVF failure	Yes	24.5 ± 31.5	0.515
	No	28.8 ± 37.2	
Blood transfusion	Yes	40.6 ± 49.6	0.535
	No	23.9 ± 26.5	

We analyzed the correlation between IL6 and hsCRP. A strong positive correlation was seen between hsCRP and IL 6 levels as shown in figure 3. The correlation between IL6 and hsCRP showed r square value of 0.853 with p value of 0.002.

Figure 3: Correlation between IL6 and hsCRP



Correlation of other acute phase reactants with IL6 and hsCRP was measured. Total WBC count was having significant correlation with IL6 and hsCRP. There was a negative correlation between IL6 and hsCRP with albumin, but was not statistically significant. Serum ferritin level did not show any significant correlation with IL6 and hsCRP.

Table 11: Correlation between different inflammatory markers

	IL6	hsCRP	WBC count	albumin	ferritin
IL6		.924	.493	−.324	.244
hsCRP	.924		.492	−.205	.061

The association between the baseline parameters and mortality was analyzed. Baseline IL6 (p value .007) , hsCRP levels (p value .038), and total leukocyte count (p value .006) was significantly different between the death and alive group.

Table 12: Parameters of alive and dead patient groups

Parameters	**Alive**	**Dead**	**p**
Age (years)	51.7 ± 11.1	61.7 ± 8.5	0.091
Diabetes Mellitus	17	2	0.916
Hypertension	33	4	0.548
BMI (kg/m^2)	21.1 ± 2.5	21.1 ± 0.6	0.984
Baseline GFR(ml/min/BSA)	5.6 ± 2.3	5.5 ± 1.5	0.944
Hb (gm/dl)	8.1 ± 1.6	8.2 ± 2.1	0.886
TC (mm^3)	8285 ± 2461	13166 ± 4743	0.006
Sodium(m mol/L)	133 ± 7	129 ± 10	0.330
Potassium (m mol/L)	4.8 ± 1.1	5.8 ± 1.6	0.117
Bicarbonate (m mol/L)	14.2 ± 4.1	11.5 ± 4.6	0.227
Albumin (g/dL)	3.7 ± 0.5	3.5 ± 0.2	0.417
Cholesterol (mg/dL)	166.7 ± 54.5	139.5 ± 50.2	0.500
Calcium (mg/dL)	7.8 ± 0.9	8.1 ± 0.8	0.539
Phosphorus (mg/dL)	5.9 ± 2.8	7.0 ± 2.1	0.474
PTH (pg/ml)	383 ± 312	223 ± 142	0.391
Ferritin (ng/ml)	401 ± 236	632 ± 302	0.379
IL6 (pgm/L)	15.7 ± 11.1	52.1 ± 47.4	0.007
hsCRP (mg/L)	24.7 ± 31.3	62.1 ± 48.2	0.038

Patient Survival rates:

Baseline IL6, hsCRP levels and total leukocyte count was significantly different between the death and alive patient groups. The receiver operating characteristic (ROC) curves of IL 6, hsCRP and total leukocyte count was done and cut off points related to mortality was determined. The best predictive value for CRP was 36.3mg/L (sensitivity 75% and specificity 81.6%). The best predictive value of IL 6 was 34.5pgm/L (sensitivity 75% and specificity 94%). The best predictive value of WBC count was 7500 (sensitivity 100% and specificity 40%). Kaplan Meier survival analysis showed there is a significant difference in survival between the patients with the different base line level of inflammatory markers.

The receiver operating characteristic (ROC) curves of IL 6 showed value of 34.5pgm/L (sensitivity 75% and specificity 94%) as best predictive cut off points related to mortality as shown in figure 4.

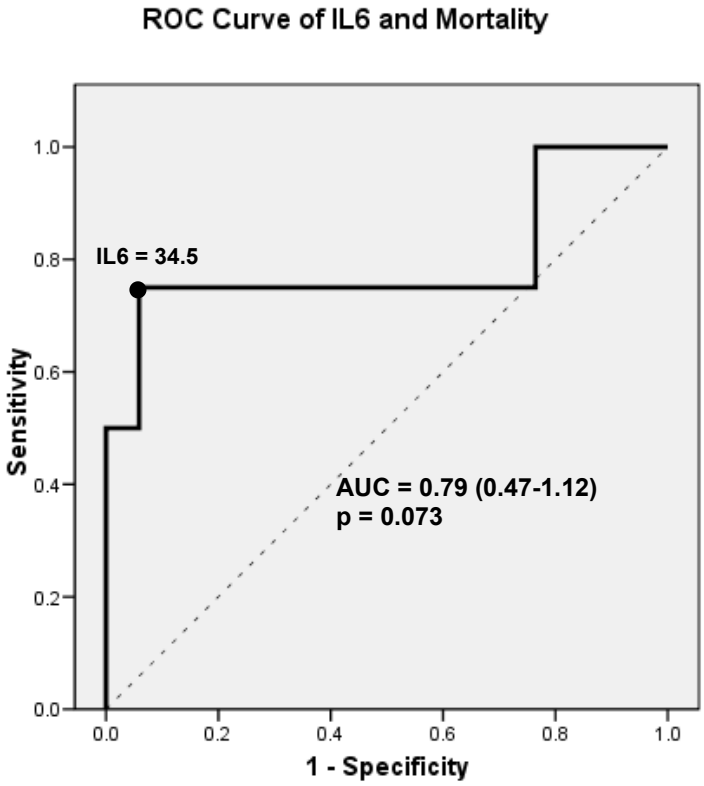


Figure 4: ROC curve of IL6 and Mortality.

Kaplan Meier survival analysis of patients with IL6 value cut off of 34.5 pgm /ml is shown in figure 5. There is a significant difference in survival between the patients with IL6 values below 34.5 pgm/ml and ≥ 34.5 pgm/ml.

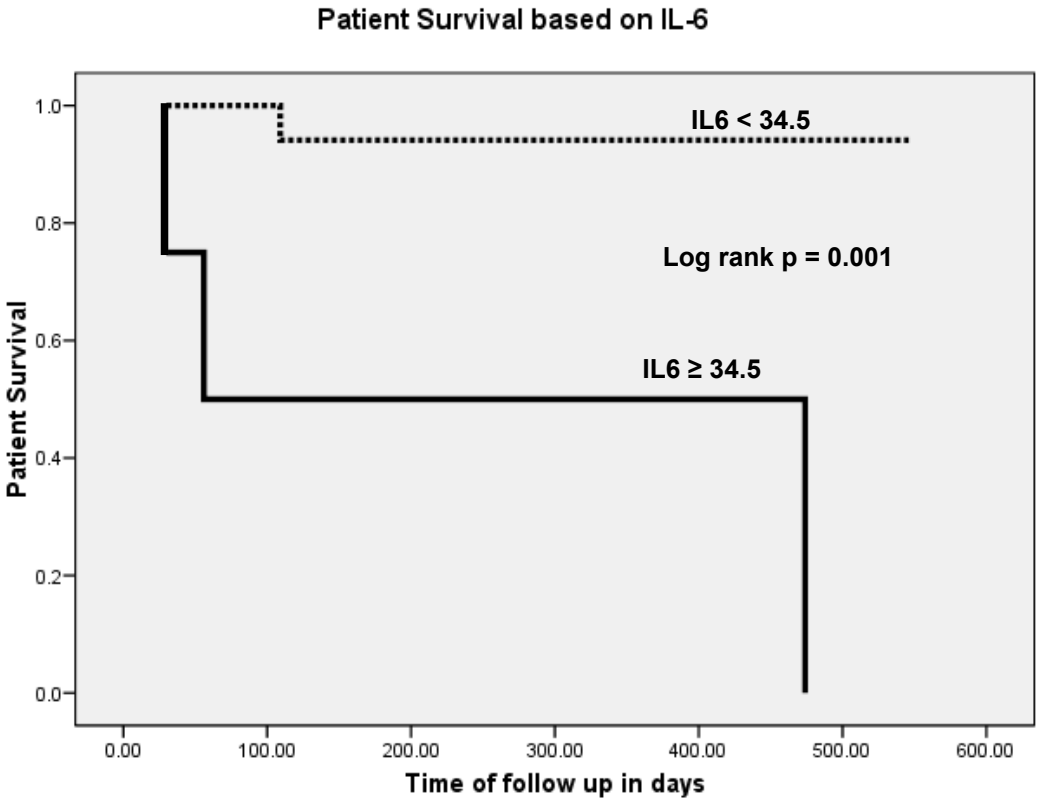


Figure 5: Patient survival based on IL6 values.

The receiver operating characteristic (ROC) curves of hsCRP showed value of 36.3mg/L (sensitivity 75% and specificity 81.6%) as best predictive cut off points related to mortality as shown in figure 6 .

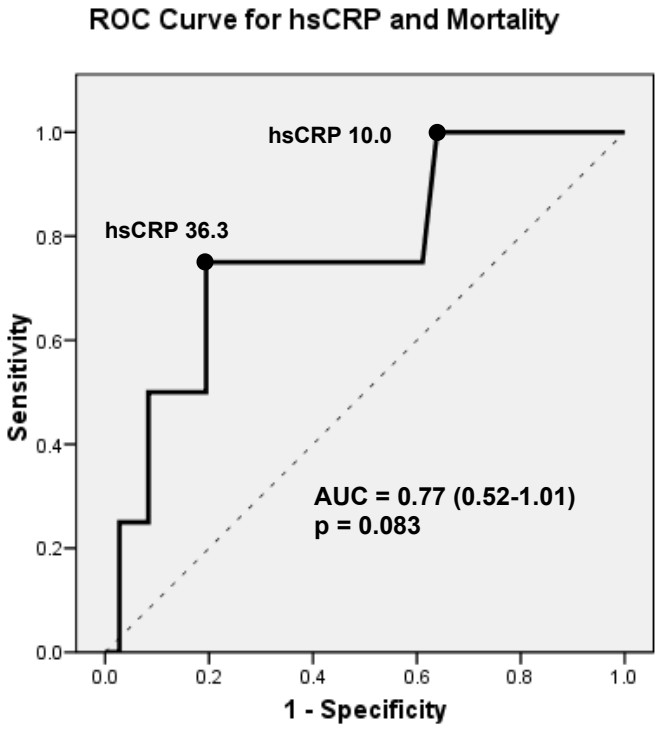


Figure 6: ROC curve for hsCRP and mortality.

Kaplan Meier survival analysis of patients with hsCRP value cut off of 36.3mg/L is shown in figure 7. There is a significant difference in survival between the patients with hsCRP values below 36.3mg/L and ≥ 36.3mg/L.

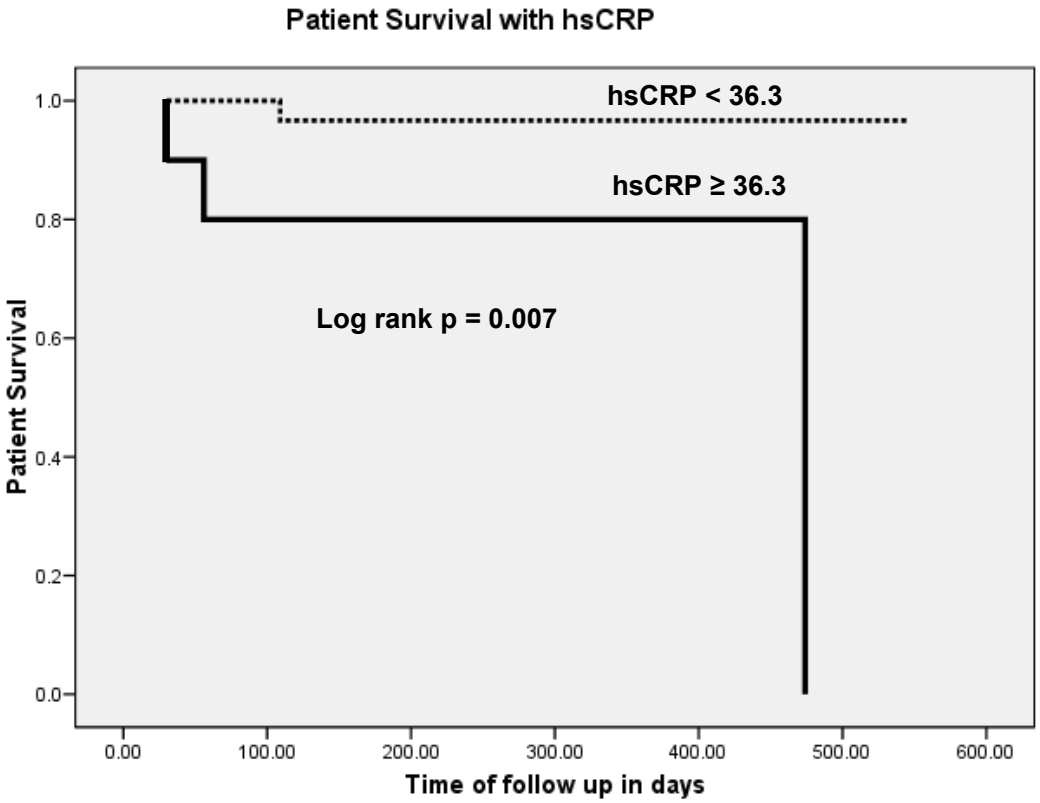


Figure 7: Patient survival with hsCRP cutoff of 36.3 mg/L.

Kaplan Meier survival analysis of patients with hsCRP value cut off of 10mg/L is shown in figure 8.

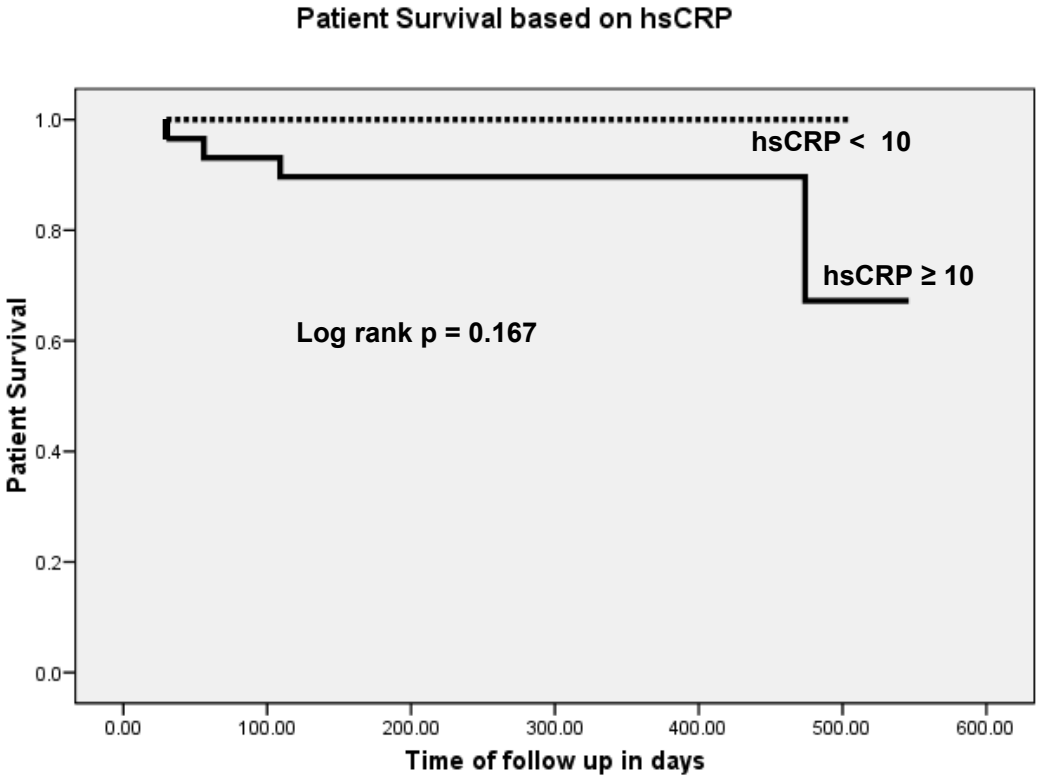


Figure 8: Patient survival with hsCRP cutoff of 10 mg/L.

The receiver operating characteristic (ROC) curves of total leukocyte count showed value of 7500 mm^3(sensitivity 100% and specificity 40%) as best predictive cut off points related to mortality as shown in figure 9.

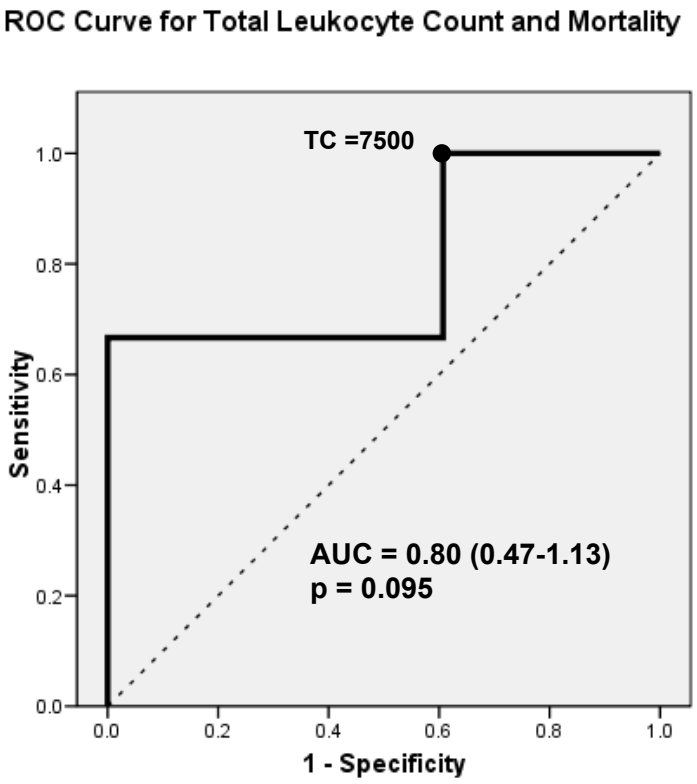


Figure 9: ROC curve for total leucocyte count and mortality

Kaplan Meier survival analysis of patients with total leukocyte cut off of 7500 mm^3 is shown in figure 10. There is a significant difference in survival between the patients with total leukocyte count below 7500 and ≥ 7500 mm^3.

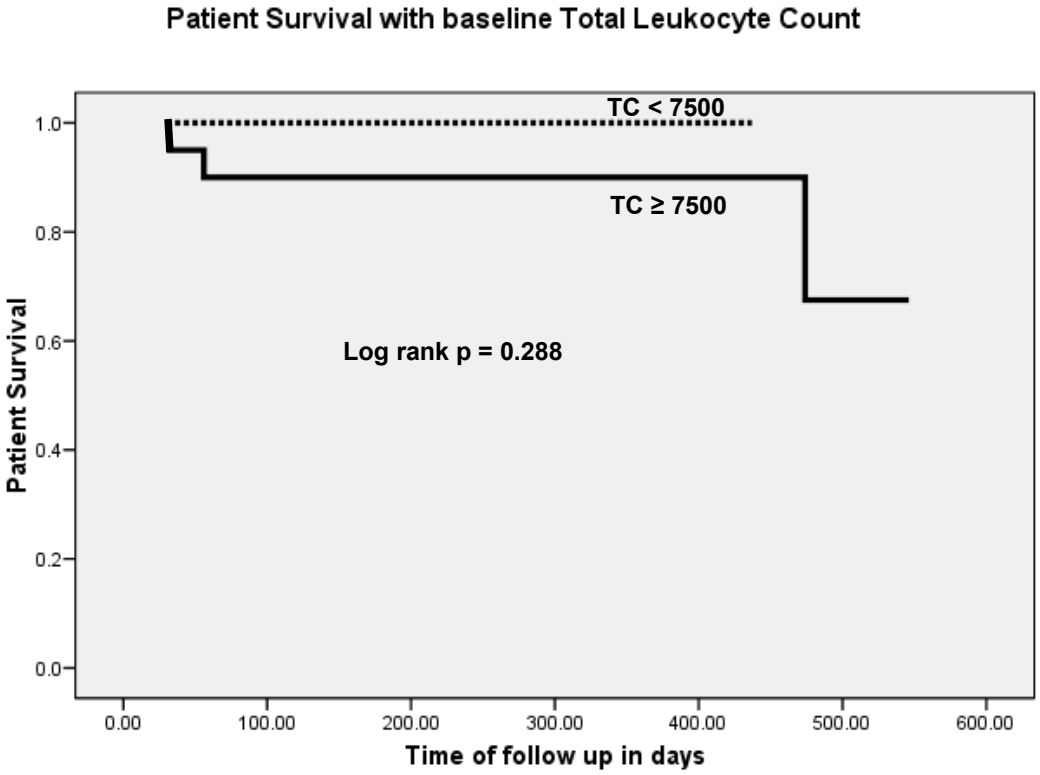


Figure 10: Patient survival with baseline leukocyte count.

Discussion

The cause for low life expectancy of patients on maintenance dialysis is multifactorial (75) . Higher age group, associated comorbid illness and increased inflammation are considered to be a major factor for decreased life expectancy.

age:

Increasing age is associated with reduced survival in patients on maintenance haemodialysis. The mean age at initiation of maintenance dialysis in our study population was 52.72 ± 11.25 years. Patients below 45 years will do better on dialysis when compared with older age groups (76). Our study showed patients who died were having higher mean age (61.7 ± 8.5) at initiation of dialysis, when compared with patient continuing on maintenance haemodialysis.

Cormorbid illness:

Associated comorbid illness is more prevalent in new patients starting on dialysis when compared with previous years (77). 55% of our patients started on maintenance dialysis were having one or more comorbid illness. Patient with other comorbid illness is associated with poor outcome on maintenance haemodialysis. End stage renal disease patients with cardiovascular disease, diabetes mellitus, and peripheral vascular disease will have more fluid overload with sympathetic over activity and increased inflammation which may be the reason for poor outcome of these patients on maintenance dialysis.

In 2007 United States Renal Data System Annual Report, diabetes mellitus was shown as the leading cause of end stage renal disease for patients initiated on maintenance dialysis which was 44%.In our study diabetic nephropathy was the native kidney disease in 47.5% of patients. Studies have shown prevalence of hypertension during the onset of dialysis is about 80% (78). In our study hypertension

was present in 92.5% of patients. Poorly controlled hypertensive patients had a greater interdialytic weight gain and poor fluid control status which is associated with increased mortality and morbidity.

Cardiovascular disease is very common in dialysis patients. In the HEMO study about 80% of patients were having some form of cardiac disease of which 40% were ischemic cardiac disease (79) . Cardiovascular disease accounts for about 50% of death in end stage renal disease. Choices for healthy outcome in caring for ESRD (CHOICE) study have shown that a large percentage of incident dialysis patients have various traditional risk factor for cardiovascular disease (80) . CHOICE study showed in patients initiated on dialysis diabetes mellitus was present in 54%, hypertension in 96%, low HDL in 33%, and increased age with mean age of about 60 years. In our study diabetes mellitus was present in 52.5% and hypertension in 92.5% and low serum HDL in 39.6% of patients. In dialysis patients the chronic inflammation in vessels can lead to accelerated atheroma formation with erosion and fissuration of plaques which can lead to rupture of plaques. The increased cardiovascular mortality in dialysis patient may be attributed to same reason.

Comorbidity index:

Various comorbid indices have been used for predicting mortality in end stage renal disease patients (81). In our study we used we used the Davies comorbidity index which have been shown to be a good semi-quantitative predictor of mortality in chronic kidney disease patients (82). Associated comorbid illness were associated with poor outcome in dialysis patients (83) .As shown in our study patients with higher grades of comorbidities was associated with higher mortality and morbidity. Further studies are needed to identify and weight the comorbidity variables for accurate risk assessment.

Inflammation:

Chronic kidney disease is a chronic inflammatory state. The inflammatory cascade can account for anorexia, decreased skeletal muscle protein synthesis and increased catabolism. These can explain the increased association of malnutrition in end stage renal disease and dialysis population. Control of chronic inflammation can improve nutritional status of these patients. There is a strong association between malnutrition and inflammation and both of which can lead to poor outcome in patients on maintenance haemodialysis.

Microinflammatory markers:

Studies have shown that about 30 to 60% of patients on dialysis have increased inflammatory markers (84). Increased inflammatory markers in dialysis patients strongly predict all cause and cardiovascular mortality(84,85). Since inflammatory markers are actively involved in pathogenesis of athero-thrombogenesis, elevated inflammatory markers should be considered as a risk factors for the same (86). Inflammatory markers are not only risk markers but also a risk factor for poor outcome in dialysis population. The cause of elevated inflammatory markers in end stage renal disease may be due to impaired clearance of uraemic toxins, associated comorbid conditions, chronic infections and other unknown factors. Our study has shown that higher baseline inflammatory markers were associated with higher mortality. There was a significant difference in survival when we compared the patients with inflammatory markers above and below the cut off value predictive of mortality. There was also a trend to higher baseline inflammatory markers in other clinical events like hypotension, LVF, pulmonary oedema, infections, AVF failure,

blood transfusions, but it was not statistically significant. This may be due to the fact that the study sample was small. Larger trials may be needed to decide on utility of these markers in predicting the morbidity in maintenance dialysis population.

IL6 and hsCRP were having good positive correlation. Other acute phase reactants did not show statistically significant correlation. The measurement of inflammatory markers can be routinely used in patients initiated on maintenance haemodialysis for risk stratification and prognostification.

Clinical events:

Hypotension was the commonest events during follow up which occurred in about 50% of patients. One or more infection episode was present in about 25% of patients during follow up. Sepsis and access related infection was the commonest cause of infection in these patients. Infections can exacerbate the preexisting chronic inflammation and can leads to poor outcome. About 40% of patients had one or more episodes of AV fistula failure. Chronic inflammation and increased athero-thrombogenesis may be the reason for increased vascular access failure in these groups of patients.

Mortality:

There was a significant difference in total leukocyte counts, IL6 and hsCRP levels between the patients who died during follow up. Baseline inflammatory markers levels can be used as predictive markers for poor outcome in patients on maintenance haemodialysis. The higher cutoff values for mortality predictor in our haemodialysis patients may be due to higher baseline chronic inflammation associated with increased infection and fluid overload in these patients.

Conclusions

Conclusions:

1. Comorbid illness is associated with poor outcome in patients initiated on maintenance dialysis
2. Presence of higher baseline inflammatory markers levels is a poor prognostic marker in maintenance dialysis patients
3. IL 6 value of more than 34.5pgm/L is predictive of mortality with sensitivity of 75% and specificity 94%.
4. HsCRP value of more than 36.3mg/L is predictive of mortality with sensitivity of 75% and specificity 81.6%.
5. There is a strong positive correlation between CRP and IL 6 levels
6. Our study highlights the fact that measurement of baseline inflammatory markers level should be considered for patients initiating on maintenance dialysis for risk stratification.

Bibliography

Bibliography:

1. Menon V, Greene T, Wang X, Pereira AA, Marcovina SM, Beck GJ, et al. C-reactive protein and albumin as predictors of all-cause and cardiovascular mortality in chronic kidney disease. Kidney Int. 2005;68(2):766-772.

2. Kimmel PL, Phillips TM, Simmens SJ, Peterson RA, Weihs KL, Alleyne S, et al. Immunologic function and survival in hemodialysis patients. Kidney Int. 1998 Jul;54(1):236-244.

3. Elhage R, Clamens S, Besnard S, Mallat Z, Tedgui A, Arnal J, et al. Involvement of interleukin-6 in atherosclerosis but not in the prevention of fatty streak formation by 17beta-estradiol in apolipoprotein E-deficient mice. Atherosclerosis. 2001 Jun;156(2):315-320.

4. Lawrence Y. Agodoa, Paul W. Eggers. Renal replacement therapy in the United States: Data from the United States renal data system. Am J Kidney Dis. 1995 Jan 1;25(1):119-133.

5. Rayner HC, Pisoni RL, Bommer J, Canaud B, Hecking E, Locatelli F, et al. Mortality and hospitalization in haemodialysis patients in five European countries: results from the Dialysis Outcomes and Practice Patterns Study (DOPPS). Nephrology Dialysis Transplantation. 2004 Jan 1;19(1):108 -120.

6. RN Foley, PS Parfrey, MJ Sarnak. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis. 1998 Nov 1;32(5):S112-S119.

7. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int. 1999 Feb;55(2):648-658.

8. Eiji Ishimura, Tetsuo Shoji, Masanori Emoto, Kouka Motoyama, Kayo Shinohara, Naoki Matsumoto, et al. Renal insufficiency accelerates atherosclerosis in patients with type 2 diabetes mellitus. Am J Kidney Dis. 2001 Oct 1;38(4):S186-S190.

9. Ross R. Atherosclerosis--an inflammatory disease. N. Engl. J. Med. 1999 Jan 14;340(2):115-126.

10. Zoccali C, Mallamaci F, Tripepi G, Cutrupi S, Parlongo S, Malatino LS, et al. Fibrinogen, mortality and incident cardiovascular complications in end-stage renal failure. J. Intern. Med. 2003 Aug;254(2):132-139.

11. Stenvinkel P. Inflammation in end‐stage renal failure: could it be treated? Nephrology Dialysis Transplantation. 2002;17(suppl 8):33 -38.

12. Bolton CH, Downs LG, Victory JG, Dwight JF, Tomson CR, Mackness MI, et al. Endothelial dysfunction in chronic renal failure: roles of lipoprotein oxidation and pro-inflammatory cytokines. Nephrol. Dial. Transplant. 2001 Jun;16(6):1189-

1197.

13. Descamps-Latscha B, Herbelin A, Nguyen AT, Roux-Lombard P, Zingraff J, Moynot A, et al. Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. J. Immunol. 1995 Jan 15;154(2):882-892.

14. Vaziri ND. Oxidative stress in uremia: nature, mechanisms, and potential consequences. Semin. Nephrol. 2004 Sep;24(5):469-473.

15. Ohtsuka T, Hamada M, Hiasa G, Sasaki O, Suzuki M, Hara Y, et al. Effect of beta-blockers on circulating levels of inflammatory and anti-inflammatory cytokines in patients with dilated cardiomyopathy. J. Am. Coll. Cardiol. 2001 Feb;37(2):412-417.

16. Witko-Sarsat V, Friedlander M, Nguyen Khoa T, Capeillère-Blandin C, Nguyen AT, Canteloup S, et al. Advanced oxidation protein products as novel mediators of inflammation and monocyte activation in chronic renal failure. J. Immunol. 1998 Sep 1;161(5):2524-2532.

17. Mimić-Oka J, Simić T, Djukanović L, Reljić Z, Davicević Z. Alteration in plasma antioxidant capacity in various degrees of chronic renal failure. Clin. Nephrol. 1999 Apr;51(4):233-241.

18. Paul JL, Sall ND, Soni T, Poignet JL, Lindenbaum A, Man NK, et al. Lipid peroxidation abnormalities in hemodialyzed patients. Nephron. 1993;64(1):106-109.

19. Drueke T, Witko-Sarsat V, Massy Z, Descamps-Latscha B, Guerin AP, Marchais SJ, et al. Iron Therapy, Advanced Oxidation Protein Products, and Carotid Artery Intima-Media Thickness in End-Stage Renal Disease. Circulation. 2002 Oct 22;106(17):2212-2217.

20. Stenvinkel P, Pecoits-Filho R, Lindholm B. Gene polymorphism association studies in dialysis: the nutrition-inflammation axis. Semin Dial. 2005 Aug;18(4):322-330.

21. Honkanen E, Grönhagen-Riska C, Teppo A, Maury C, Meri S. Acute-Phase Proteins during Hemodialysis: Correlations with Serum Interleukin-1β Levels and Different Dialysis Membranes. Nephron. 1991;57(3):283-287.

22. Pereira BJ, Snodgrass B, Barber G, Perella C, Chopra S, King AJ. Cytokine production during in vitro hemodialysis with new and formaldehyde- or renalin-reprocessed cellulose dialyzers. J. Am. Soc. Nephrol. 1995 Oct;6(4):1304-1308.

23. Memoli B, Minutolo R, Bisesti V, Postiglione L, Conti A, Marzano L, et al. Changes of serum albumin and C-reactive protein are related to changes of interleukin-6 release by peripheral blood mononuclear cells in hemodialysis patients treated with different membranes. Am. J. Kidney Dis. 2002

Feb;39(2):266-273.

24. Ismail N, Becker BN, Hakim RM. Water Treatment for Hemodialysis. Am J Nephrol. 1996;16(1):60-72.

25. Bambauer R, Walther J, Jung W. Ultrafiltration of Dialysis Fluid to Obtain a Sterile Solution during Hemodialysis. Blood Purif. 1990;8(6):309-317.

26. Vanholder R, Ringoir S, Dhondt A, Hakim R. Phagocytosis in uremic and hemodialysis patients: a prospective and cross sectional study. Kidney Int. 1991 Feb;39(2):320-327.

27. Jaar BG, Hermann JA, Furth SL, Briggs W, Powe NR. Septicemia in diabetic hemodialysis patients: comparison of incidence, risk factors, and mortality with nondiabetic hemodialysis patients. Am. J. Kidney Dis. 2000 Feb;35(2):282-292.

28. Pastan S, Soucie JM, McClellan WM. Vascular access and increased risk of death among hemodialysis patients. Kidney Int. 2002 Aug;62(2):620-626.

29. Kadiroglu AK, Kadiroglu ET, Sit D, Dag A, Yilmaz ME. Periodontitis is an important and occult source of inflammation in hemodialysis patients. Blood Purif. 2006;24(4):400-404.

30. Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, et al. Effect of Dialysis Dose and Membrane Flux in Maintenance Hemodialysis. New England Journal of Medicine. 2002 Dec 19;347(25):2010-2019.

31. Kalantar-Zadeh K, Kopple JD, Block G, Humphreys MH. A malnutrition-inflammation score is correlated with morbidity and mortality in maintenance hemodialysis patients. Am. J. Kidney Dis. 2001 Dec;38(6):1251-1263.

32. Pecoits‐Filho R, Lindholm B, Stenvinkel P. The malnutrition, inflammation, and atherosclerosis (MIA) syndrome – the heart of the matter. Nephrology Dialysis Transplantation. 2002 Nov 1;17(suppl 11):28 -31.

33. Mehrotra R, Kopple JD. Nutritional management of maintenance dialysis patients: why aren't we doing better? Annu. Rev. Nutr. 2001;21:343-379.

34. Kalantar-Zadeh K, Block G, McAllister CJ, Humphreys MH, Kopple JD. Appetite and inflammation, nutrition, anemia, and clinical outcome in hemodialysis patients. The American Journal of Clinical Nutrition. 2004;80(2):299 -307.

35. Lee P, Peng H, Gelbart T, Wang L, Beutler E. Regulation of hepcidin transcription by interleukin-1 and interleukin-6. Proc. Natl. Acad. Sci. U.S.A. 2005 Feb 8;102(6):1906-1910.

36. Weinstein DA, Roy CN, Fleming MD, Loda MF, Wolfsdorf JI, Andrews NC. Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. Blood. 2002 Nov

15;100(10):3776-3781.

37. Jurado RL. Iron, infections, and anemia of inflammation. Clin. Infect. Dis. 1997 Oct;25(4):888-895.

38. Bloembergen WE. Cardiac disease in chronic uremia: epidemiology. Adv Ren Replace Ther. 1997 Jul;4(3):185-193.

39. Levey AS. Controlling the epidemic of cardiovascular disease in chronic renal disease: where do we start? Am. J. Kidney Dis. 1998 Nov;32(5 Suppl 3):S5-13.

40. Galis ZS, Sukhova GK, Lark MW, Libby P. Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest. 1994 Dec;94(6):2493-2503.

41. Goldsmith DJ, Covic A. Coronary artery disease in uremia: Etiology, diagnosis, and therapy. Kidney Int. 2001 Dec;60(6):2059-2078.

42. Geng YJ, Henderson LE, Levesque EB, Muszynski M, Libby P. Fas is expressed in human atherosclerotic intima and promotes apoptosis of cytokine-primed human vascular smooth muscle cells. Arterioscler. Thromb. Vasc. Biol. 1997 Oct;17(10):2200-2208.

43. Honda H, Qureshi AR, Heimbürger O, Barany P, Wang K, Pecoits-Filho R, et al. Serum albumin, C-reactive protein, interleukin 6, and fetuin a as predictors of malnutrition, cardiovascular disease, and mortality in patients with ESRD. Am. J. Kidney Dis. 2006 Jan;47(1):139-148.

44. DeFronzo RA, Alvestrand A, Smith D, Hendler R, Hendler E, Wahren J. Insulin resistance in uremia. J Clin Invest. 1981 Feb;67(2):563-568.

45. Alvestrand A. Carbohydrate and insulin metabolism in renal failure. Kidney Int. Suppl. 1997 Nov;62:S48-52.

46. Moller DE. Potential role of TNF-alpha in the pathogenesis of insulin resistance and type 2 diabetes. Trends Endocrinol. Metab. 2000 Aug;11(6):212-217.

47. Hak AE, Pols HAP, Stehouwer CDA, Meijer J, Kiliaan AJ, Hofman A, et al. Markers of Inflammation and Cellular Adhesion Molecules in Relation to Insulin Resistance in Nondiabetic Elderly: The Rotterdam Study. J Clin Endocrinol Metab. 2001 Sep 1;86(9):4398-4405.

48. Fernandez-Real J, Vayreda M, Richart C, Gutierrez C, Broch M, Vendrell J, et al. Circulating Interleukin 6 Levels, Blood Pressure, and Insulin Sensitivity in Apparently Healthy Men and Women. J Clin Endocrinol Metab. 2001 Mar 1;86(3):1154-1159.

49. Tripepi G, Mallamaci F, Zoccali C. Inflammation Markers, Adhesion Molecules, and All-Cause and Cardiovascular Mortality in Patients with ESRD: Searching for the Best Risk Marker by Multivariate Modeling. Journal of the American Society

of Nephrology. 2005 Mar 1;16(3 suppl 1):S83 -S88.

50. van Riemsdijk-van Overbeeke IC, Baan CC, Hesse CJ, Loonen EH, Niesters HG, Zietse R, et al. TNF-alpha: mRNA, plasma protein levels and soluble receptors in patients on chronic hemodialysis, on CAPD and with end-stage renal failure. Clin. Nephrol. 2000 Feb;53(2):115-123.

51. Bemelmans MH, Gouma DJ, Buurman WA. Influence of nephrectomy on tumor necrosis factor clearance in a murine model. J. Immunol. 1993 Mar 1;150(5):2007-2017.

52. Lowrie EG, Lew NL. Death risk in hemodialysis patients: the predictive value of commonly measured variables and an evaluation of death rate differences between facilities. Am. J. Kidney Dis. 1990 May;15(5):458-482.

53. Bologa RM, Levine DM, Parker TS, Cheigh JS, Serur D, Stenzel KH, et al. Interleukin-6 predicts hypoalbuminemia, hypocholesterolemia, and mortality in hemodialysis patients. Am. J. Kidney Dis. 1998 Jul;32(1):107-114.

54. Mears E. Outcomes of continuous process improvement of a nutritional care program incorporating serum prealbumin measurements. Nutrition. 1996 Aug;12(7-8):479-484.

55. Barisani D, Pelucchi S, Mariani R, Galimberti S, Trombini P, Fumagalli D, et al. Hepcidin and iron-related gene expression in subjects with Dysmetabolic Hepatic Iron Overload. J. Hepatol. 2008 Jul;49(1):123-133.

56. Stenvinkel P, Heimbürger O, Paultre F, Diczfalusy U, Wang T, Berglund L, et al. Strong association between malnutrition, inflammation, and atherosclerosis in chronic renal failure. Kidney Int. 1999 May;55(5):1899-1911.

57. Papanicolaou DA, Wilder RL, Manolagas SC, Chrousos GP. The Pathophysiologic Roles of Interleukin-6 in Human Disease. Annals of Internal Medicine. 1998 Jan 15;128(2):127 -137.

58. Huber SA, Sakkinen P, Conze D, Hardin N, Tracy R. Interleukin-6 exacerbates early atherosclerosis in mice. Arterioscler. Thromb. Vasc. Biol. 1999 Oct;19(10):2364-2367.

59. Fukuzawa M, Satoh J, Sagara M, Muto G, Muto Y, Nishimura S, et al. Angiotensin converting enzyme inhibitors suppress production of tumor necrosis factor-alpha in vitro and in vivo. Immunopharmacology. 1997 Apr;36(1):49-55.

60. Stenvinkel P, Andersson P, Wang T, Lindholm B, Bergström J, Palmblad J, et al. Do ACE-inhibitors suppress tumour necrosis factor-alpha production in advanced chronic renal failure? J. Intern. Med. 1999 Nov;246(5):503-507.

61. Diomede L, Albani D, Sottocorno M, Donati MB, Bianchi M, Fruscella P, et al. In vivo anti-inflammatory effect of statins is mediated by nonsterol mevalonate products. Arterioscler. Thromb. Vasc. Biol. 2001 Aug;21(8):1327-1332.

62. Seliger SL, Weiss NS, Gillen DL, Kestenbaum B, Ball A, Sherrard DJ, et al. HMG-CoA reductase inhibitors are associated with reduced mortality in ESRD patients. Kidney Int. 2002 Jan;61(1):297-304.

63. Fellström BC, Jardine AG, Schmieder RE, Holdaas H, Bannister K, Beutler J, et al. Rosuvastatin and Cardiovascular Events in Patients Undergoing Hemodialysis. N Engl J Med. 2009 4;360(14):1395-1407.

64. Wanner C, Krane V, März W, Olschewski M, Mann JFE, Ruf G, et al. Atorvastatin in patients with type 2 diabetes mellitus undergoing hemodialysis. N. Engl. J. Med. 2005 Jul 21;353(3):238-248.

65. Boaz M, Smetana S, Weinstein T, Matas Z, Gafter U, Iaina A, et al. Secondary prevention with antioxidants of cardiovascular disease in endstage renal disease (SPACE): randomised placebo-controlled trial. Lancet. 2000 Oct 7;356(9237):1213-1218.

66. Omata M, Higuchi C, Demura R, Sanaka T, Nihei H. Reduction of neutrophil activation by vitamin E modified dialyzer membranes. Nephron. 2000 Jul;85(3):221-231.

67. Tepel M, van der Giet M, Statz M, Jankowski J, Zidek W. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. Circulation. 2003 Feb 25;107(7):992-995.

68. Yamada K, Fujimoto S, Tokura T, Fukudome K, Ochiai H, Komatsu H, et al. Effect of sevelamer on dyslipidemia and chronic inflammation in maintenance hemodialysis patients. Ren Fail. 2005;27(4):361-365.

69. Rammohan M, Kalantar-Zadeh K, Liang A, Ghossein C. Megestrol acetate in a moderate dose for the treatment of malnutrition-inflammation complex in maintenance dialysis patients. J Ren Nutr. 2005 Jul;15(3):345-355.

70. Don BR, Kim K, Li J, Dwyer T, Alexander F, Kaysen GA. The effect of etanercept on suppression of the systemic inflammatory response in chronic hemodialysis patients. Clin. Nephrol. 2010 Jun;73(6):431-438.

71. Yang B, Baughman S, Sullivan JT. Pharmacokinetics of anakinra in subjects with different levels of renal function. Clin. Pharmacol. Ther. 2003 Jul;74(1):85-94.

72. Sitter T, Bergner A, Schiffl H. Dialysate related cytokine induction and response to recombinant human erythropoietin in haemodialysis patients. Nephrol. Dial. Transplant. 2000 Aug;15(8):1207-1211.

73. Eknoyan G, Beck GJ, Cheung AK, Daugirdas JT, Greene T, Kusek JW, et al. Effect of dialysis dose and membrane flux in maintenance hemodialysis. N. Engl. J. Med. 2002 Dec 19;347(25):2010-2019.

74. Davies SJ, Phillips L, Naish PF, Russell GI. Quantifying comorbidity in

peritoneal dialysis patients and its relationship to other predictors of survival. Nephrology Dialysis Transplantation. 2002 Jun 1;17(6):1085 -1092.

75. Collins AJ, Foley RN, Herzog C, Chavers BM, Gilbertson D, Ishani A, et al. Excerpts From the US Renal Data System 2009 Annual Data Report. Am J Kidney Dis. 2010 Jan;55(1 Suppl 1):S1-A7.

76. Mailloux LU, Bellucci AG, Napolitano B, Mossey T, Wilkes BM, Bluestone PA. Survival estimates for 683 patients starting dialysis from 1970 through 1989: identification of risk factors for survival. Clin. Nephrol. 1994 Aug;42(2):127-135.

77. Miskulin D, Bragg-Gresham J, Gillespie BW, Tentori F, Pisoni RL, Tighiouart H, et al. Key Comorbid Conditions that Are Predictive of Survival among Hemodialysis Patients. Clinical Journal of the American Society of Nephrology. 2009 Nov 1;4(11):1818 -1826.

78. Zucchelli P, Santoro A, Zuccala A. Genesis and control of hypertension in hemodialysis patients. Semin. Nephrol. 1988 Jun;8(2):163-168.

79. Cheung AK, Sarnak MJ, Yan G, Berkoben M, Heyka R, Kaufman A, et al. Cardiac diseases in maintenance hemodialysis patients: Results of the HEMO Study. Kidney Int. 2004 Jun;65(6):2380-2389.

80. Longenecker JC, Coresh J, Powe NR, Levey AS, Fink NE, Martin A, et al. Traditional cardiovascular disease risk factors in dialysis patients compared with the general population: the CHOICE Study. J. Am. Soc. Nephrol. 2002 Jul;13(7):1918-1927.

81. van Manen JG, Korevaar JC, Dekker FW, Boeschoten EW, Bossuyt PMM, Krediet RT. How to adjust for comorbidity in survival studies in ESRD patients: a comparison of different indices. Am. J. Kidney Dis. 2002 Jul;40(1):82-89.

82. Caravaca F, Martín MV, Barroso S, Ruiz B, Hernández-Gallego R. Do inflammatory markers add predictive information of death beyond that provided by age and comorbidity in chronic renal failure patients? Nephrology Dialysis Transplantation. 2006 Jun;21(6):1575 -1581.

83. Khan I. Influence of coexisting disease on survival on renal-replacement therapy. The Lancet. 1993 2;341(8842):415-418.

84. Yeun JY, Levine RA, Mantadilok V, Kaysen GA. C-Reactive protein predicts all-cause and cardiovascular mortality in hemodialysis patients. Am. J. Kidney Dis. 2000 Mar;35(3):469-476.

85. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C. Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int. 1999 Feb;55(2):648-658.

86. Pepys MB, Hirschfield GM. C-reactive protein: a critical update. J. Clin. Invest. 2003 Jun;111(12):1805-1812.

Appendices

PROFORMA

(A) PATIENT CHARACTERISTICS

Name:

Age (years): Height: Weight: BMI:

Sex: M = 0, F = 1 Smoking : Y/N

Address: Phone:

Occupation:

Hospital number:

Native Kidney Disease

Renal biopsy done: Yes/No

e GFR – Daily Urine output :

Associated comorbidites pre HD

DISEASE	Y/N	DURATION	DISEASE	Y/N	DURATION
DIABETES MELLITUS			COPD		
HYPERTENSION			INFECTIONS		
LVF			MALIGNANCY		
IHD			OTHERS		
PVD					
CONNECTIVE TISSUE DIS					

DRUGS: - ASPIRIN-Y/N, ACE/ARB-Y/N, STATINS-Y/N,

ERYTHROPOETIN- Y/N (weekly dose), PARENTERAL IRON (dose)

Date of initiation of Hemodialysis: Thrice/Twice Weekly, Duration:

	Y/N	DURATION		Y/N	DURATION
JUGULAR CATH			FEMORAL CATH		
SUBCLAVIAN CATH			AV FISTULA/GRAFT		

Lab Parameters	Pre HD	3rd month	6th month	9th month	12th month	15th month	18th month
HB							
Total count							
TP/Serum Albumin							
Lipids							
Na/K/Hco3							
Bicarbonate							
Ca/Po4/							
PTH							
Serum Ferritin							
Uric acid							
Hs CRP							
IL-6							

Intercurrent clinical events

EVENTS	Y/N	EPISODES	EVENTS	Y/N	EPISODES
HYPOTENSION			ACCESS THROMBOSIS		
LVF			ACCESS INFECTION		
CORONARY DISEASE			INFECTIONS		
ARRYTHMIAS			OTHERS		
CVA					
BLOOD TRANSFUSION					

Notes -